ECONOMIC BOTANY

Devoted to Applied Botany and Plant Utilization

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... Tannin from Wilcox Bark ... Industrial Raw Materials of Animal Origin ... Chemicals from Oranges ... Sea ... Natural ... Oak ...

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Semi-Popular Article

USEFUL NATIVE PLANTS IN THE AMERICAN SOUTHWESTERN DESERTS. *A. Krochmal, S. Paur and P. Duisberg* 3

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Useful Native Plants in the American Southwestern Deserts[1]

In addition to yuccas, creosote bush, prickly pear cactus, agaves and the wax-producing candelilla bush, all of which have already yielded to some commercial exploitation, scores of other plants listed in this article also grow in the region and contain industrial potentialities.

A. KROCHMAL[2], S. PAUR[3] AND P. DUISBERG[4]

The Southwestern desert and semi-arid region of the United States spreads over almost a quarter million square miles, comprising much of Arizona, southern New Mexico and parts of Texas and California. Many very unusual and weird plants, with the ability to survive under the most rigorous conditions, can be found growing in this vast area. At tremendous cost man has been able to pick out promising sites in the region where he can harness water for irrigation and produce high-yielding and high moisture-consuming crops.

These irrigated areas constitute about two percent of the total land area and are responsible for much of the income derived from the region. The remaining 98% of the land is used largely for grazing to support livestock. During periods of high rainfall, overgrazing does not present too large a problem. Unfortunately, during the past ten years the average yearly rainfall has been far below normal, and many areas have been so overgrazed that much of the valuable cover of grass has been completely removed, and undesirable weeds and shrubs have begun to replace the grass.

As a result of the strong emphasis placed on irrigation farming and on livestock production, the great potential natural resources of the native desert plants have been overlooked. With the filling of irrigation dams by silt, the lowering of water tables and the possibility of range-lands becoming depleted, serious problems are presenting themselves. It would therefore seem wise to look into the vast so-called "wasteland" to determine whether some of the many plants now growing there could be either utilized in their present surroundings or removed for production under cultivation.

Although very few of these desert plants have been studied to determine their chemical constituents, the outlook for this type of research appears promising, with work along these lines being carried on at several southwestern colleges and experiment stations. This type of research is often slow to yield results, but a brief summary of some of the work that has been accomplished so far through colleges, experiment stations, federal agencies and private individuals is presented here.

[1] The material in this article, and the bibliography, have been assembled with emphasis on listing the maximum number of plants that offer possible utilization for economic purposes. The material may be considered supplementary to that previously presented in ECONOMIC BOTANY (25*a*).

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Yuccas

Utilization of yucca fiber has occupied a place of extreme importance, not only in the older civilizations of the ancient southwestern Basket-makers and Cliff dwellers, but also among the Indians of New Mexico and Arizona who use the fiber for making brooms, brushes, baskets, ropes, cords, belts and mats.

During the first World War, yucca fiber from New Mexico and Texas was successfully used to make 80 million pounds of bagging and burlap, but this promising development was not continued after the war. Interested German scientists, however, collected seed in this country and developed varieties suitable to their growing conditions. The fiber produced from these plantings was found to be the equal of wool in the manufacture of textiles. The project was discontinued because of the failure of the Germans to develop a cheap means of separating the fiber from the other leaf material.

A shortage of fibers during the second World War renewed interest in yucca, and a factory was built at Kingman, Arizona, to extract the fiber. This, too, proved a failure because of poor planning and lack of information.

About the same time New Mexico A and M College began a series of experiments to determine the quality of yucca fibers. It was found that the strength of the fibers varies with the species and that the two native yuccas are equal in strength to the best imported fibers. The people engaged in this problem found that there are areas in the Southwest where yucca is present in sufficient quantities to provide a commercial source of fiber.

The most important drawback to the production of yucca fiber on a large scale has been the lack of a satisfactory method for separating the fiber from the leaf residue. A machine has recently been developed that appears to do an efficient job.

A considerable amount of saponins is also obtained from the pulp after fiber removal. The residue left after the saponins are removed may have value as a livestock feed.

Creosote Bush (*Larrea divaricata* Cav.)

Of the many common shrubs that cover the southwestern region, creosote bush can be found in the greatest abundance. It has been used by the Indians for the treatment of almost as many diseases as has penicillin.

Up to 1942 the shrub was virtually unknown to the chemist. At that time scientists at the University of Minnesota successfully extracted a material known as "nordihydroguaiaretic acid", or N.D.G.A., which proved to be a remarkable antioxidant (rancidity retarder) for fats and oils. This material is now being produced commercially, and its value is reported to be about $45 a pound. Further work was started at New Mexico A and M in 1945 by one of the authors to determine the N.D.G.A. content of the creosote bushes growing in New Mexico. It was found that the leaves contain considerably more than had been supposed, but commercial yields were much lower, indicating room for improvement in the purification process. As little as .001 percent N.D.G.A. added to fats and oils will prevent rancidity many months.

Further experimentation indicates that after removal of the resinous materials, the extracted leaf contains 16% protein, or as much as alfalfa, and could be fed to livestock. Possible utilization of the wood and the presence of an essential oil are other worth-while fields for further research.

Cactus (*Opuntia* spp.)

For many years ranchers have used prickly-pears, with the spines removed, as a source of stock feed in periods of drought. Recently a Texas industrial concern has begun to evaluate the eco-

FIG. 1. *Yucca elata*. Southern Arizona, maximum growth.

nomic possibilities of processing prickly pears as a feed source.

Gums of various types have been obtained from cholla (*Opuntia fulgida* Eng.) and saguaro (*Cereus giganteus* Eng.). Some of these gums are used in Mexico as a size and cloth-stiffner in local textile mills. Mucilages with good adhesive properties have also been obtained from cactus gums. Oils have been extracted from a number of species of *Opuntia* and *Cereus*.

A high yield of almost pure cellulose has been obtained from a species of Sardinian cactus (*Opuntia ficus-indica* L.). Several alkaloids have been isolated from various cacti, 11 of them from peyote, or mescal (*Lophophora Williamsii* Lem.) alone. Various novelties are made from the dried hollow cylinder of wooden meshes of ocotillo (*Fouquieria splendens* Engelm.).

The Indians of the Southwest have long used the fruits and leaves of cacti as an important source of food, the fruits of the saguaro being utilized to make a syrup, wine and vinegar, and dried for storage. From the barrel cactus (*Echinocactus wislezenii* Eng.) both jellies and the famed cactus candy are obtained.

It should be kept in mind that cacti are valuable scenic resources, and their utilization should be regulated.

Agaves

Included among the agaves are several members commonly called "century plants", *i.e.*, mescal, maguey and lechuguilla. A drink called "pulque" is made from the fermented sap of *Agave*, while the alcoholic drink mescal, or tequilla, and industrial alcohol are made from the central stems and leaf bases. Some species are commercial sources of the fibers ixtile, sisal and henequen, from which ropes, sacking, cordage, bushes and carpets are made.

It has been suggested that agaves could be used in the manufacture of emulsions, pectins, toilet preparations, boiler compounds, vitamin C complex, glucosides and saponins, and as a precursor for cortisone.

Mesquite (*Prosopis*)

Invasion of range land by mesquite is costing ranchers millions of dollars each year. Range management experts from several experiment stations have been assigned the task of developing methods for destruction of the plant.

As an incentive for its removal, the Texas Forest Service Laboratory is attempting to develop useful products from mesquite. The plant will probably be considered as a source of charcoal, tannins, stock feed, alcohol, acids, plastics, wall-board, and sugars by wood-hydrolysis. An extender for phenol-formaldehyde plastics has been successfully made from mesquite wood flour. Trinkets and novelties can also be made from the very hard reddish wood. Mesquite gum is a source of uncommon sugars, such as L-arabinose and D-galactose, and is used to some extent in the manufacture of mucilages and gum-drops.

Candelilla (*Pedilapthus pavanis*)

The candelilla bush contains 3.5% to 5% wax, used in the manufacture of floor pastes, sealing wax, candles, phonograph records, electrical insulators, water-proof boxes and fabrics. Most of the extraction plants are in Mexico, one in Texas.

Bear Grass (*Nolena microcarpa*)

Each plant of bear-grass will yield from 30 to 75 pounds of green leaf. The Indians have long used this fiber for weaving baskets and mats. Bear grass is now being harvested in Mexico and New Mexico, and used as a substitute for broom-straw in the manufacture of brooms.

Cucurbits

At Texas A and M and New Mexico A and M colleges the seeds of three native gourds are under test to determine their

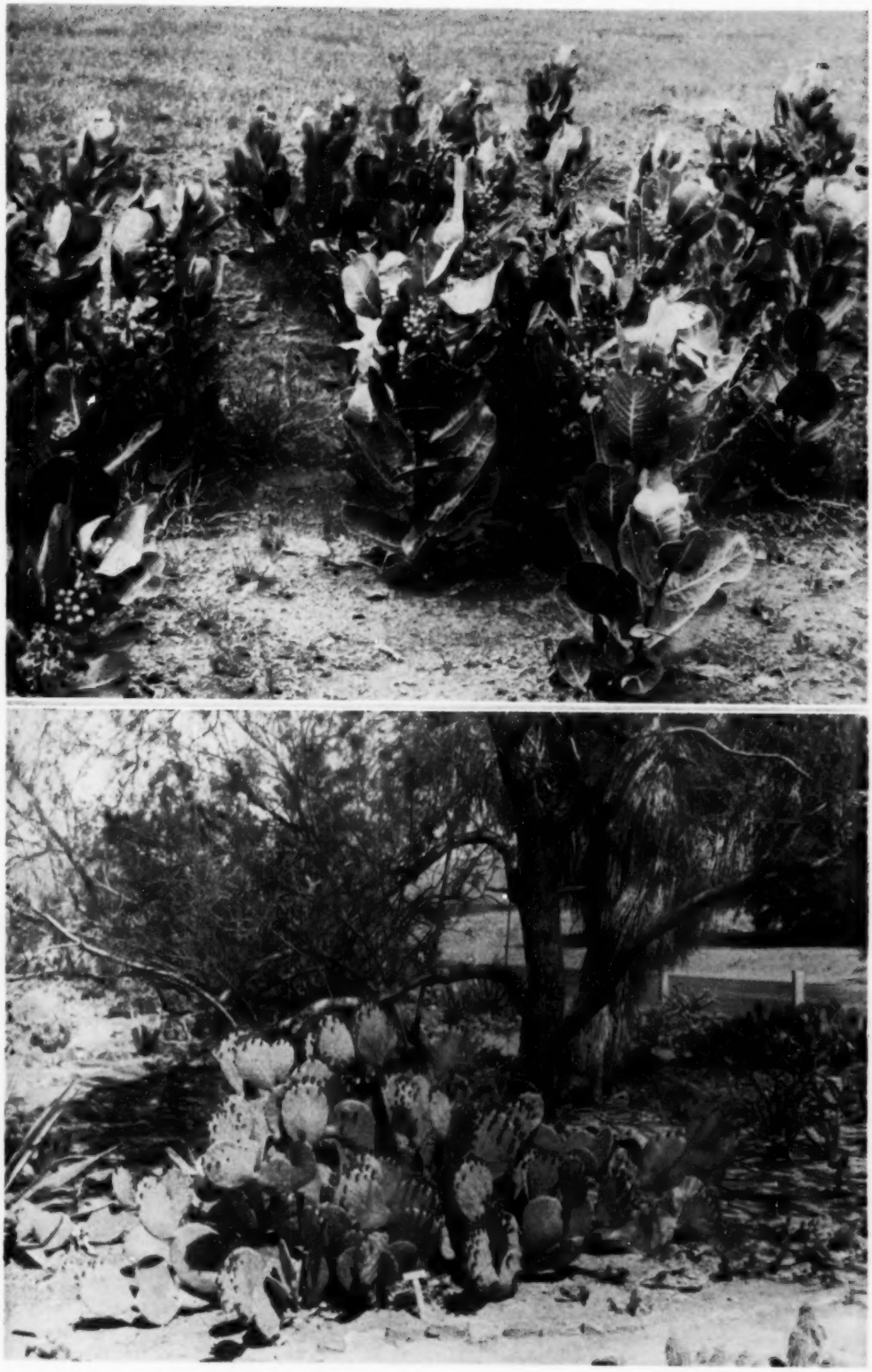

FIG. 2 (*Upper*). *Asclepias latifolia*, a potential rubber source.
FIG. 3 (*Lower*). *Opuntia* sp., the wide-spread prickly-pear.

value for high-protein concentrate feeds, and the quality and quantity of oil produced in such seeds. These plants are *Cucurbita foetidissima, C. palmata* and *C. digitata,* and their seeds contain approximately 29, 30 and 25 percent oil. The latter two are classified as drying-oils, like linseed oil, while the first has semi-drying properties like cottonseed oil.

Devil's Claw (*Martynia parviflora*)

The New Mexico A and M College has attempted to evaluate the seeds of devil's claw as an oil source, and has found them to contain about 36% oil. This semi-drying oil resembles cottonseed oil and sunflower oil, and it is believed it could be very satisfactorily substituted for cottonseed oil in the manufacture of salad oils and shortenings.

Jojoba (*Simmondsia chinensis*)

Approximately 50% of the weight of the seeds of this plant is a liquid wax, with a potential importance for the lubrication of delicate instruments, typing-ribbon inks and polishes, and may be useful in the production of cosmetics.

Canaigre (*Rumex hymenosephalus*)

The roots of this plant average 25% tannin or better, and the Indians have used them for many years as such a source. They were commercially shipped to the East and Europe in the late 1880's and the early 1900's. Research now being conducted at the Texas Experiment Station, the U.S.D.A. station at Sacaton, Arizona, and Eastern Regional Laboratories in Philadelphia indicate that this plant produces a most satisfactory tannin.

Although the outlook appears very bright for many of the foregoing plants to be utilized on a commercial scale, it must be strongly emphasized that in most cases additional research is needed to determine whether the plants can be produced economically, and to thoroughly work out production and manufacture methods. It is clear that results based on team-work of agronomists, horticulturists and chemists will not be available immediately, but will take several years of effort.

Drugs

Aplopappus nuttallii T.&G.F.N. From the roots the Hopi Indians made a tea for treating coughs (39).

Apocynum cannabinum L. Indian hemp, Dog-bane. From the roots a cardiac stimulant similar but inferior to digitalin is obtained (39).

Arctostaphylos pungens HBK. Pointleaf manganita. The leaves are used as a remedy for stomach trouble in Arizona (39).

Arctostaphylos uva-ursi Spreng. Common bearberry, Kinnikinick. Has been used for treating disorders of the urinary tract and as a substitute for tobacco (26, 39).

Berberis spp. Barberry. It is believed that many of the species contain berberine (6, 39).

Buddleja sessiliflora L. Has been used medicinally in Mexico (39).

Ceanothus integerrinos L. Deerbush. An infusion of the bark is used as a tonic (6, 39).

Cercis accidentalis L. Redbud. The astringent bark of the tree has been used as a remedy for diarrhea and dysentery (39).

Chenopodium ambrosioides L. Spanish tea, Mexican tea. A strong anti-helmintic is made from liquid distilled from the leaves and stems (39).

Cichorium intybus L. Chicory, Succory. Roots are used as an adulterant of or substitute for coffee, particularly cultivated forms (19, 20).

Clematis ligusticifolia Nutt. The Indians used this plant as a remedy for sore throats and cold, and crushed leaves were placed in the nostrils of tired horses to revive them (6, 39).

Commelina spp. Dayflower. A few species are said to be able to stop the flow of blood (39).

Cordylanthus wrightii Nutt. Clubflower. Hopi Indians used this plant to bleach their skin (39).

Croton corymbulosus Rothr. Chaparral tea, Encinilla. A domestic medicine has been made from the leaves in Texas (6, 24, 39).

Croton texensis Klotzsch. Skunkweed. Has been used by the Hopis as an eyewash and emetic, and by other Indians to relieve paralysis and as a purgative (26, 39).

Datura meteloides Dunal. All parts of this plant are narcotic; it is eaten by many Indians to induce visions (6, 26, 39).

Datura stramonium L. Jimson-weed, Thorn-apple. Same as above. Contains many alkaloids, notably atropine (daturine). All parts of the plant are poisonous (15, 16, 17, 22, 23, 26).

Ephedra torreyana Wats. Mormon tea. A decoction is used as a diuretic (6, 26).

Erigeron canadensis L. Horseweed. Used as a remedy for diarrhea and dysentery (6, 39).

Euphorbia hirta L. Spurge. Used in treating asthma and bronchitis, and is an official drug plant (39).

Eysenhardtia spp. Kidneywood. The wood of several species is fluorescent when soaked in water, and has been reported to be diuretic (39).

FIG. 4. Foreground, *Cucurbita digitata*. Note spherical fruit at right. Background, *C. palmata*.

Flourensia cernua DC. Tarbush. In northern Mexico the leaves and heads are sold in the drug markets for treating indigestion under the name "hojase" or "hojasen" (6, 39).

Fouquierra splendens Engelm. Ocotillo. To relieve tiredness and fatigue the Apache Indians bathe in a decoction of the roots, and also apply the powdered roots to painful swellings (6, 39).

Fremontodendron californicum Torr. Flannel-bush, Fremontia. An infusion

of the bark is used to relieve irritations of the throat (39).

Gaillardia pinnatifida Torr. The Hopi Indians have been reported to use this plant as a diuretic (39).

Glycyrrhiza lepidota L. Licorice. The sweet roots contain almost as much glycyrrhizin, used by druggists and confectioners, as do the imported roots of *G. glabra* L. (39).

Grindelia squarrosa Willd. Gumweed, Tarweed. This species and others of the genus are official drug plants and are administered in asthma, used as an anti-spasmodic and stomachic, and externally to relieve the irritation caused by poison ivy (39).

Heliotropium curassavicum L. Heliotrope, Turnsole. The Pima Indians applied the dried roots in powdered form to sores and wounds (39).

Heracleum lanatum Michx. Cow-parsnip. The roots have been used to control epilepsy, and appear to be somewhat stimulative and carminative (6, 39).

Heuchera spp. Alum-root. The astringent root-stocks were used by early settlers to cure diarrhea caused by drinking alkali water (6, 39).

Hymenopappus lugens Greene. The Hopis were reported to use the roots for treating toothache and as an emetic (39).

Jatropha macrorrhiza Benth. The roots are said to be strongly purgative (6, 39).

Krameria parvifolia Benth. Range ratany. An infusion of the twigs serves the Papago Indians in treating sore eyes (6, 39).

Lantana camara L. An alkaloid resembling quinine in action has been found in this plant (39).

Larrea divaricata L. Creosote bush. Contains about 9% of an anti-oxidant called nordihydroguaiaretic acid (NDGA) which is extracted from the leaves and green stems. It is used as a preservative for fats and oils.

Ligusticum porteri Koch. Lovage. The aromatic roots are used to treat coughs and colds. Called " coughroot " in the drug market (39).

Marrubium vulgare L. Horehound. Used as a confection for easing sore throats and checking coughs (39).

Monarda spp. Bee-balm. A few species have been employed in domestic medicine (39).

Nepeta cataria L. Catnip. Although this is an official drug plant, it has no therapeutic value other than being a mild aromatic. It has been used, however, as a mild stimulant, a tonic and to quiet nervous patients (39).

Nicotiana attenuata Torr. Tobacco. The dried leaves are smoked by many New Mexicans of Spanish origin (6, 26).

Nicotiana glauca Graham. Tree tobacco. The plants contain an alkaloid which has been reported more effective in killing certain species of aphids than nicotine compounds (39).

Nicotiana trigonophylla Dunal in DC. Tobacco. Smoked by Indians for ceremonial events (39).

Opuntia spp. Prickly pear, Indian fig. The heated young " leaves " have been employed to treat various types of rheumatism and other body pains; used as a poultice (6, 26). Mexicans value the green portion as a salad.

Perezia wrightii Gray and *P. nana* Gray. The roots of both these species yield pipitzahoic acid which is sometimes used as an indicator of alkalinity (39).

Plantago fastigiata Morris and *P. purshii* R. & S. Indian wheat. The seeds of these species have been used as a substitute for psyllium seed (6, 39).

Pluchea sericea Nutt. Marsh fleabane. The Pima Indians have been reported to make an infusion of the herbage to treat sore eyes (39).

Populus fremontii Wats. and *P. tremuloides* Michx. Fremont's cottonwood, Quaking aspen, Trembling aspen, Quiver-leaf. The inner bark is used

FIG. 5 (*Upper*). *Cucurbita foetidissima.*
FIG. 6 (*Lower*). *Martynia parviflora*, the Devils Claw. Note the curved fruits.

by the Indians as an anti-scorbutic (6, 39).

Prosopis odorata Torr. & Frem. Fremont screwbean. The Pima Indians use the inner bark to treat wounds (6, 39).

Ranunculus sceleratus L. Buttercup. It is said that beggars have used the acrid juice to induce sore-formation (39).

Rhus glabra L. Smooth sumac, Scarlet sumac. The fruits have astringent properties and are official in the U. S. Pharmacopoeia (6, 39).

Robinia neomexicana Gray. New Mexico locust. This plant has been reported to be used as an emetic and in treating rheumatism by the Hopis (6, 39).

Salix spp. Willow. From the bark of several species the drug salicin is obtained, which is used as a tonic and antiperiodic, and has febrifugal properties (6, 39).

Selloa glutinosa Spreng. Used in Mexico for treating diarrhea (39).

Solanum eleagnifolium Cav. Bullnettle. The Pima Indians added the crushed berries of this plant to milk in making cheese. An enzyme, similar to papain, has been obtained from the plant (26, 39).

Sonchus oleraceus L. Sow-thistle. The gum obtained from the juice is said to be strongly cathartic (39).

Sphaeralcea spp. Globe-mallow. Used by the Hopi Indians for treating disorders of the bowels (39).

Verbascum thapsus L. Mullein, Flannel-plant. The dry leaves have served as a substitute for tobacco (26).

Verbesina encelioides Cav. Crown-beard. Both the Hopis and early white settlers used this plant to treat boils and other skin diseases (39).

Wyethia scabra Hook. Mules ears. Used by the Navajos and Hopis as an emetic but considered dangerous by them (39).

Xanthium commune Britt. Cocklebur. Indians use leaves boiled in water to relieve diarrhea. A poultice made of the leaves supposedly cures rattlesnake bites (26).

Foods, Flavorings, Seasonings

Acacia greggii Gray. Catclaw acacia. From the pods of this plant Arizona Indians made a meal for use in cakes and mush (6, 10, 26, 39).

Agave spp. Century-plant. Fermentation of the fleshy leaves yields potent liquors, pulque and mescal. The fibers sisal and henequen are obtained from the plants. Navajos roasted the crowns for several days to produce a nourishing food (6, 10, 39).

Acanthochiton wrightii Torr. Eaten by Hopis as greens or dried and stored (10, 39).

Allium spp. Several species are used by the various tribes for food and seasoning (6, 10, 39).

Amaranthus spp. Pigweed. Both the young leaves and stems of several species have been used as greens, and the mature seeds for meal (6, 10, 16, 39).

Arctostaphylos pungens HBK. Pointleaf manzanita. An excellent jelly is made from the unripe fruits (6, 39).

Arundo donax L. Giant reed. Young shoots are sometimes eaten by Indians.

Asclepias galioides HBK. Milkweed. The immature pods have been eaten by the Indians, as well as the young greens (6, 10).

Astragalus ceramicus Sheld. Milk vetch. In the spring Hopi children eat the sweet roots of this species (39).

Atriplex spp. Salt-bush, Orach. The Indians made a meal from the parched seeds and used the leaves and young shoots as greens (6, 10, 39).

Atriplex canescens Pursh. Fourwing saltbrush. The Hopi Indians are reported as using the ashes of this plant as a substitute for baking powder (39).

Calochartus spp. Mariposa lily, Globe tulip, Butterfly lily. Both Hopis and

Navajos eat the large fleshy bulbs of many species (6, 10, 39).

Chenopodium spp. L. Lambs-quarter, Pigweed, Poulette, Chou grass. From the seeds of many species the Indians made mush and cakes, and ate the leaves for greens (6, 10, 26, 39).

Cichorium intybus L. Chicory. The roots, particularly of cultivated forms, are eaten under the name "witloof", while the leaves may be cooked or eaten raw (39).

Cleome spp. Beeweed, Spiderflower. Some species have been eaten by Indians as greens (6, 10, 39).

Cucurbita foetidissima HBK. Buffalo gourd, Fetid wild pumpkin. Both New Mexico and Arizona Indians make a type of mush from the seeds. The whole fruits were dried for winter use (6, 26, 39).

Cycloloma atriplicifolium Spreng. Winged pigweed. A type of mush and cakes are made from the ground seeds (39).

Cymoptenus bulbosus L. The dried leaves are used to flavor chile and beans (6, 39).

Daucus pusillus Michx. Wild carrot. The roots are eaten raw or boiled by the Navajos (10).

Descurainia spp. Tansy-mustard. A few species are used by the Indians for greens and in making pinole from the parched and ground seeds (39).

Echinocereus spp. Hedge-hog cactus. Both pulp and rind have been used by Indians in making conserves (6, 10).

Ephedra spp. Mormon tea. From the dried flowers and stems palatable tonic beverage is made. Source of ephedrine (8, 39).

Helianthus annuus L. Sunflower. The seeds of this and other species are eaten by the Indians (6, 10, 26, 39).

Heracleum lanatum Michx. Cow parsnip. Indians have been reported as eating the young stems and leaves (6, 39).

Hoffmanseggia densiflora Benth. Hog-potato. The tubers make excellent hog food and, after roasting, good human food (6, 10, 26, 39).

Humulus americanus Nutt. Hop. Used by the Apache Indians. Their name for it means "make bread with it" (6, 39).

Hydrophyllum occidentale Gray. Western squaw lettuce. Leaves are used as salad greens (39).

Iris missouriensis Nutt. Blue flag. A substitute for coffee is made from roasted and ground seeds (39).

Lomatium spp. Raf. Biscuitroot. The edible roots of several species have been eaten raw, cooked or ground into flour (39).

Lycium pallidum Miers. Rabbit thorn. The ripe fruits are either eaten raw or cooked by Indians (6, 10, 26).

Mentha spp. Peppermint. The aromatic leaves were used by the Hopis for flavoring mush and also used as a stomachic (10, 26, 39).

Monarda menthaefolia Graham. Bee-balm, Fern mint. The Hopis often cultivated this plant as a green, and dried it for winter use. The dried leaves of this species and *M. pectinata* L. are used as a seasoning for beans and stews (10, 26, 39).

Monolepis nuttalliana R. & S. Patata, Povertyweed. The Indians made pinole from the seeds, and ate the vegetative parts as greens; the roots were also eaten (10, 39).

Oenothera trichocalyx Nutt. Evening-primrose. Used as a potherb (6, 39).

Opuntia spp. Prickly-pear. After the spines are burned off, the joints may be used as cattle feed, containing about 8% protein. Young joints, stems and fruits of several species are eaten raw by Indians and Mexicans (6, 10, 26, 39).

Pectis papposa Harv. & Gray. The Indians in New Mexico use the flowers to flavor meat (10, 39).

Perideridia gairdneri L. Wild-caraway. The Indians used the tuberous roots as food, while the seeds served for seasoning (39).

Phaseolus acutifolius Gray. Tepary beans. The small white beans have been used by both Indians and the pioneers. Somewhat less palatable than *P. vulgaris*.

Phoradendron californicum Nutt. Mistletoe. The berries are dried and stored, or boiled and eaten directly by the Papagos and other Indians (10, 39).

Physalis spp. Ground-cherry. The berries have been used in making preserves, and have been eaten cooked and raw by the Indians (6, 10, 26, 39).

Phytolacca americana L. Poke-weed, Scoke, Garget, Pigeon-berry. The succulent young shoots make excellent greens but must be thoroughly cooked (39).

Pinus edulis Engelm. Pinon pine, Nut pine. The fruit are widely used by the Indians, particularly the Navajos. They are also sold throughout the United States, often under the name "Indian Nuts" (6, 10, 26, 39).

Poliomintha incana Torr. The flowers are used by the Hopis for seasoning, and the vegetative parts eaten raw or cooked (10, 26, 39).

Portulaca oleracea L. Common purslane. Used by the Indians and others as a salad green (6, 10, 26, 39).

Prosopis juliflora D. Common mesquite, Honey mesquite, Screw bean, Algaroba. From the sweet pods of this plant a type of meal is made (pinole) by the Pima Indians and others, and fashioned into cakes. An alcoholic beverage is also made from the fermented pinole. The gummy exudation secreted from the bark is used to mend pottery, make candy and furnish a black dye. A substitute for rennet, used to curdle milk, is obtained from the inner bark (6, 10, 26, 39).

Prosopis odorata Torr. & Frem. Fremont screwbean. Used by the Indians for food very much in the same way as the common mesquite (6, 10, 26, 39).

Prunus virginiana L. Common chokecherry. The fruit is eaten either raw or cooked, or made into preserves (6, 10, 26, 39).

Pteridium aquilinum L. Bracken fern, Pasture brake, Hog brake. Both roots and young fronds have been used for food (39).

Quercus spp. Oak. The acorns of most species have been used for food by many Indian tribes (6, 10, 26, 39).

Rumex crispus L. Curled dock, Curly-leaf dock. Sometimes used as a salad green (6, 26, 39).

Rumex hymenosepalus Torr. Canaigre. The leaves, petioles and roots have been eaten by Indians (10, 26, 39, 40).

Stanleya pinnata Pursh. Desertplume. This plant has been used as a potherb, and a mush has been made from its seeds by the Indians (26, 39).

Solanum jamesii Torr. and *S. fendleri* L. Wild potato. The tubers of both species have been used by Hopi and other Indians in making yeast and are cooked in saline clay. The tubers are like the cultivated potato, but smaller (6, 10, 26, 39).

Suaeda spp. Seepweed, Sea-blite. The Pima and other Indians ate the young plants as greens and made pinole from the seeds (10, 26, 39).

Tecoma stans HBK. (*Stenolobium stans* Seem.). Trumpet-bush, Yellow-bells. The roots are used in Mexico for making a type of beer, and also medicinally (39).

Thelesperma longpipes Gra. Used in New Mexico as a substitute for tea under the name "cota" (6, 39).

Tradescantia spp. Spiderwort. The Indians have used this plant for salad greens (10, 26, 39).

Tradescantia pinetorum Greene. Spiderwort. The Indians reportedly eat the tuberous roots (39).

Vitis arizonica Engelm. Canyon grape,

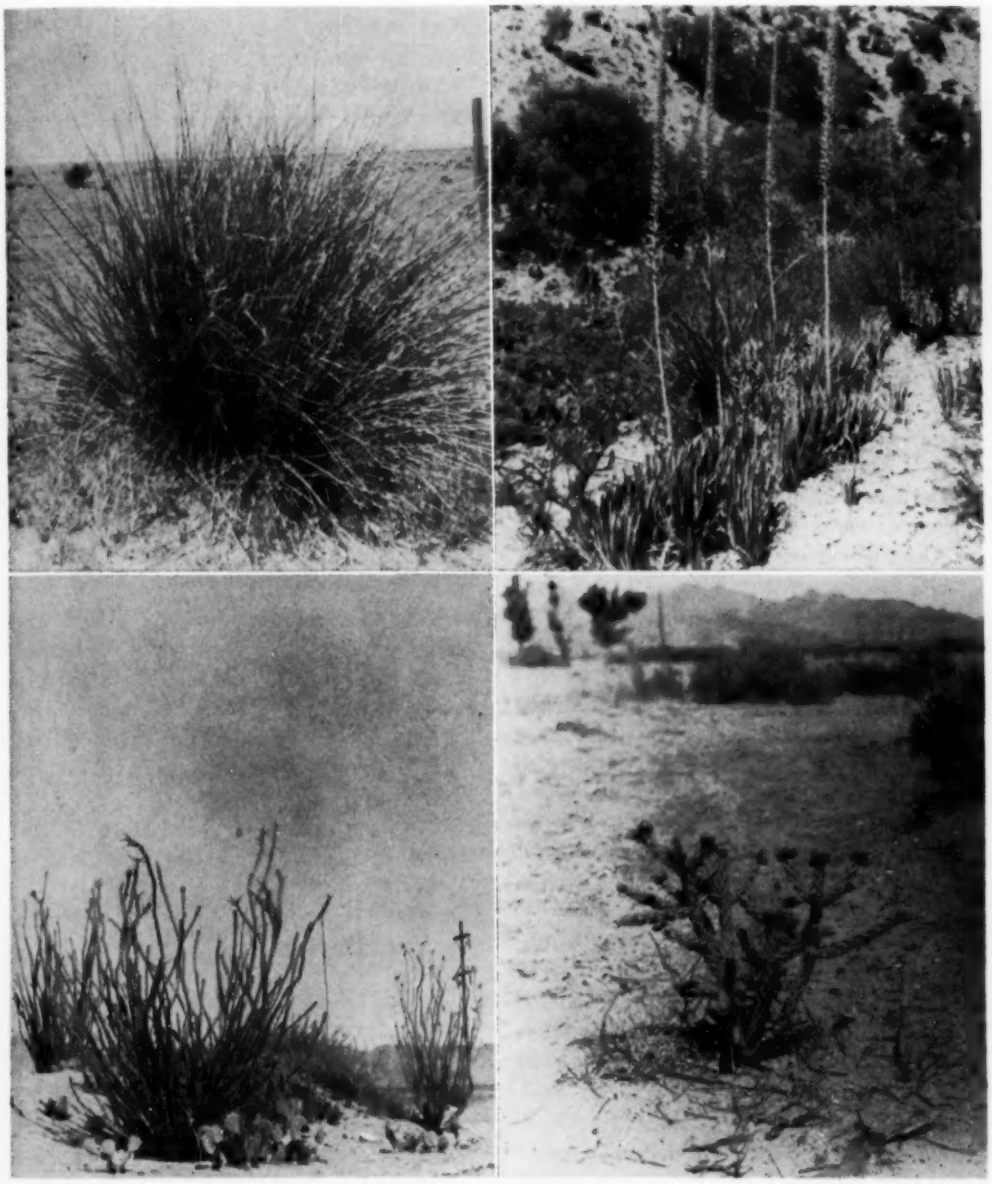

FIG. 7 (*Upper left*). *Nolina texana* in an ungrazed area eight miles east of Marfa, Texas.

FIG. 8 (*Upper right*). *Agave lechugilla*, used for a fiber source. Reputed to be poisonous to stock, causing a form of sleeping sickness.

FIG. 9 (*Lower left*). *Fouquieria splendens* Engelm., Ocotillo or Devil's walking stick, used for carving and as a fence in southwestern areas.

FIG. 10 (*Lower right*). *Opuntia fulgida* Engelm., the cholla, whose purple-red blossoms are a familiar sight to residents of desert areas.

Wild grape. The fruit is eaten fresh, or dried for raisins, by the Pueblo Indians. The ancient cliff dwellers used the fruit (10, 39).

Yucca spp. Soapweed, Beargrass, Spanish-bayonet. All parts of the flower stalks are eaten by the Indians, either raw or boiled. The large fruits of some species are ground into meal, dried and eaten raw or roasted. Soap is made from the pounded leaves. The fruits are sometimes fermented to produce an alcoholic beverage (39).

Fibers

Abutilon incanum Link. Rope is made in Mexico from the stem fiber (39).

Agave spp. American aloe. Excellent fibers are obtained from several species of this genus, particularly *A. lechugilla* which is the source of a fiber used for brushes and as a waste fiber for upholstery tow (6, 26, 39).

Apocynum spp. Indian hemp. The bark of several species has served the Indians as cordage.

Linum lewisii Pursh. Flax. The long fibers have reportedly been made into cordage by western Indians (39).

Martynia parviflora Wooten. Devils claw. Indians have used the split mature pods in weaving block designs in their baskets (6, 39).

Nolina microcarpa Wats. Beargrass. The fibers obtained from the leaves have been used by the Indians for making mats. Several small companies harvest these plants as a substitute for broom corn. The plants contain 48% fiber (6, 39).

Parryella spp. The Hopi Indians make baskets and brooms from this plant (39).

Populus fremontii Wats. Fremont cottonwood. The twigs are used by the Pima Indians for basket material (39).

Prosopis juliflora DC. Common mesquite, Honey mesquite, Screw bean, Algaroba. The Indians make a coarse fabric and baskets from the inner bark (39).

Rhus trilobata Rehd. Squawbush. The pliable stems are used in basket weaving by Indians (39).

Salix spp. Willow. The twigs furnish the Pima Indians basket material (39).

Sesbania exaltata Raf. Cory. Indians obtained excellent fibers from the stems (6, 47).

Stipa vaseyi Scribn. Porcupine grass. The fibers have been used for making brooms and brushes (26).

Yucca glauca Nutt., *Y. baccata* Torr. and *Y. elata* L. Soapweed, Spanish bayonet. The Indians make baskets, mats, sandals and rope from the excellent fibers in the leaves. Rope and paper have been made commercially from a few of these species (6, 39).

Gums and Resins

Actinea richardsoni Hook. From the bark and roots a substitute for chewing gum is made in New Mexico (39).

Apocynum cannabinum L. Dogbane, Indian hemp. From the dried milky fluid in the stems a substitute for chewing gum is made (39).

Bumelia rigida Gray. Buckthorn. Children in Texas chew the gum exuded from the bark (39).

Coursetia microphylla Gray. An orange colored lac is often found on the stems of this plant, caused by an infestation of an insect member of the genus *Tachardia*. The Papago Indians used the lac to seal jars of saguaro syrup (39).

Larrea divaricata Cav. Creosote bush. The leaves and small stems contain 15–17.5% ether-extracted resins. Varnishes can be made from this resin. Indians used the material to mend pottery and fix arrow points.

Opuntia fulgida Engelm. Cholla. The gum obtained from this plant is sold in Tucson, Arizona, and is used in Mexico as a size or stiffening. The

gum contains L-arabinose, D-galactose and glucosonic acid.

Prosopis juliflora DC. Common mesquite, etc. A gum similar to gum arabic is obtained from this plant. Contains same organic compounds as above.

Juniperus monosperma Engelm. Juniper pitch. Chewed by the Indians instead of commercial chewing gum (26, 39).

Plantago spp. Indian wheat, Plantago. When wet the seeds become mucilaginous (39).

Sphaeralcea spp. False-mallow, Globemallow. The Hopis used the mucilaginous stems as a substitute for chewing gum.

Insecticides

Anthemis cotula L. Mayweed, Stinking chamomile, Dog fennel. The dried powdered flowers have been reported to be very effective against fleas, bedbugs and flies. The leaves also have insecticidal properties (6, 39).

Artemisia spp. Wormwood. The aromatic oil obtained from several species is supposed to be insect-repellant (6).

Croton texensis Muell. & Arg. Skunkweed. Has been used as a household insecticide by Indians in New Mexico (26).

Rhinanthus rigidus L. Yellow-rattle. Has been reported to be used as an insecticide (39).

Oils

Apodanthera undulata Gray. Melonloco. The seeds contain 30% oil.

Camelina microcarpa Andrz. False-flax. An oil similar to linseed oil is extracted from the seeds (39).

Cucurbita digitata Gray. Large quantities of a drying oil have been obtained from the seeds.

Cucurbita foetidissima HBK. Buffalo gourd, Missouri gourd, Fetid wild pumpkin. The seeds contain about 27% oil with semi-drying qualities.

Cucurbita palmata Wats. Coyote melon. The seeds yield about 30% of a drying oil.

Helianthus annuus L. Sunflower. From the seeds of the cultivated varieties high quantities of a semi-drying oil are obtained, used as salad oil and for hair-dressing (6, 10, 26, 39).

Juniperus communis L. Common juniper. An aromatic oil obtained from the fruits and wood is used in flavoring gin and in various patent medicines (6, 39).

Martynia parviflora Wooten. Devils claw. The mature seeds yield 36% oil when pressed. The oil resembles both sunflower and cottonseed oil (6, 21).

Reverchonia arenaria Gray. The Indians used the seeds for oiling their grinding stones (39).

Ricinus communis L. Castor bean. The seeds yield castor oil, used medicinally and for lubricating oil, in paints and varnishes (39).

Stillingia sylvatica L. Queen's-delight, Queen's-root. The seeds have yielded up to 30% oil. A resin, an oil and a glucoside have been extracted from the roots (25).

Xanthium commune Britt. Cocklebur. The oil extractable from the burrs can be used in paints and varnishes (26).

Essential Oils

Opuntia spp. Prickly pear, Indian fig. An essential oil is extracted from the yellow and pink flowers of several species and made into perfume.

Pectis papposa Harv. and Gray. The leaves contain over 1% essential oils, on a dry weight basis.

Yucca baccata Torr. A perfume is prepared commercially from the blossoms.

Pigments

Alnus spp. Alder. A pale red dye is obtained from the bark of several species (9).

Berberis repens Lindl., *B. haemotocarpa*

Wooten. Oregon grape, Algerita. The roots of both species contain a brilliant yellow dye.

Cercocarpus spp. Mountain mahogany. The Hopis get a red-brown dye from the bark and use it to dye leather (39).

Chrysothamnus spp. Rabbitbrush. A yellow dye is obtained from the flowers of several species and a green dye from the inner bark (6, 26, 39).

Cleome serrulata L. Rocky Mountain beeplant, Stinking clover. A black dye is obtained when the entire plant is boiled in water (6, 26).

Ephedra spp. Mormon tea. The Navajos obtain a red dye from this plant.

Galium spp. Bedstraw, Cleavers. A dark red dye is obtained from the roots of several species (6).

Helianthus annuus L. Sunflower. Purple and black dyes are extracted from the seeds of this and other species by the Indians for use in baskets, textiles and painting their bodies. A yellow dye is obtained from the leaves (26, 39).

Juniperus spp. Juniper, Cedar. An olive-brown dye is extracted from the berries of several species.

Krameria parviflora Benth. Range ratany. The Papago Indians obtained a dye from the roots to color wool and other materials (39).

Lithospermum spp. Gromwell, Puccoon. The Indians obtained a purple dye from the roots (6, 39).

Pectis angustifolia Torr. The Hopi Indians extracted a dye from this plant (39).

Rhus trilobata Fern. Squawbush. The Indians used the berries as a mordant in dyeing (39).

Rivina humilis L. Rouge-plant, Pigeonberry. A red dye is obtained from the fruits (39).

Rumex hymenosepalus Torr. Canaigre. A mustard-colored dye has been obtained from the roots.

Thelesperma megapotamicum Spreng. A yellow dye is furnished by the flowers, and a brown dye from other parts of the plant, used on baskets and clothing (26, 39).

Latex

Actinea richardsoni Hook. Colorado rubber-plant. Sap contains small quantities of rubber (6, 39).

Amsonia hirtella Standl. On extraction the entire plant yields about two and one-half percent rubber (9).

Asclepias spp. Milkweed, Silkweed. Almost all milkweeds contain some rubber. *A. erosa* Torr. has produced as high as five and one-half percent rubber when grown under cultivation (9).

Chrysothamnus nauseosus Gray. Rabbitbrush. Also *C. paniculatus* Gray and *C. vescidiflorus* Hook. Sources of small amounts of latex (39).

Funastrum cynanchoides Schlecht. Gum vine. Some samples have yielded almost four percent latex (9).

Parthenium incanum HBK. Mariola. Has small amounts of latex, while *P. argentatum* Gray (guayule) has a high enough latex content to make profitable commercial production in Arizona, Texas and California (9, 39).

Solidago canadensis L. Goldenrod. This species contains about four percent latex (9).

Tecoma stans HBK. or *Stenolobium stans* Seem. Yellow-bells, Trumpet-bush. Almost four percent latex was obtained from this bush (9).

Saponins

Agave spp. American aloe. Saponins have been obtained from several species.

Cucurbita spp. Gourds. The fruits of several species contain saponins and have been used as a substitute for soap by the Indians.

Phytolacca americana L. Pokeweed, Scoke, Garget, Pigeonberry. Both roots and berries contain saponins (18, 39).

Sapindus drummondi (H.&A.). Soapberry. The fruits have been used by the Indians as a form of soap in washing clothes (39).

Saponaria officinalis L. Bouncing Bet, Soapwort. Both roots and leaves contain saponins and lather easily in water (39).

Yucca spp. Soapweed. A type of soap and a laxative are made from the saponin-rich roots (39).

Tannins

Acacia spp. Tannins can be extracted from the bark of a few species (6, 39).

Arctostaphylos uva-ursi L. Bearberry. Both leaves and bark contain tannins (6).

Erodium spp. Heronbill. It has been reported that the roots of several species contain tannins (6, 39).

Geranium spp. Cranesbill. The roots of some species contain tannins (6).

Rhus spp. Sumac. Both roots and bark of a few species contain tannins (6, 39).

Rubus spp. Brambles. Tannins are obtained from roots and bark of several species (6).

Rumex hymenosepalus Torr. The roots contain 35–42% tannins. Probably the most promising wild tannin source in the Southwest. Now being produced commercially in Arizona, and the subject of research by State and Federal agencies (6, 10, 26, 39, 40).

Salix exigua virens. Coyote willow. A solution made from bark soaked in water is used to tan hides (36).

Waxes

Fouquieria splendens Engelm. Ocotillo. An excellent belt dressing is made from the wax that coats the stems (6, 39).

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Utilization Abstract

Tomato. An annotated bibliography on the use of the tomato, disregarding nomenclature, origin, development, etc., indicates that our modern appreciation of this fruit came, not from Mexico to which it had spread by the time of the Conquest from its original home in northwestern South America, but by way of the Italians and French; it also suggests that the French took over the use from the Italians and that the latter may have become acquainted with it from the Turks, or at least from peoples in the Levant. Nearly 500 citations make up the bibliography, divided into various geographical groupings, not including, however, either Central or South America. Regarding this discrimination, the author states: "There seems to be adequate justification for this omission. These areas are, from all evidence, the home of the tomato both wild and cultivated; its use in these areas is quite ancient. Thus, the history of the use of the tomato in most of these areas is a very different problem from the one which necessarily would be the focus for the rest of the bibliography, namely, the history of the use of the tomato among peoples to whom it was introduced, fully developed as a food plant, in comparatively recent times. Certainly, the problem of research is very different". (G. A. McCue, *Ann. Mo. Bot. Garden 39*(*4*): *289–348. 1952*).

Effect of Temperature and Duration of Storage on Quality of Stored Sweetpotatoes[1]

For the six varieties studied, storage temperatures in the narrow range of 55 to 60° F. were found to be most suitable.

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The sweetpotato, unlike most of our other crop plants, is of tropical origin. Some provision, therefore, must be made in cool climates for keeping the roots in good condition from one growing season to the next for propagation as well as for food. During the necessarily extensive storage period, suitable environment must be provided for proper maintenance of life activities. Storage temperature is a crucial factor in the environment, since it determines to a great degree the character and rate of physiologic responses and the possibility and extent of decay.

Since sweetpotatoes are sensitive to cold, it is very important to have information on the effect of long-time storage at temperatures only slightly below the optimum for good storage. This paper reports the effect of continuous storage for three, five and seven months in 1948, 1949 and 1950 at 50°, 55° and 60° F., a range extending below and above a possible optimum. The maximum storage period used in this work was longer than that reported by many other workers and is about as long as any commercial storage.

The effects of holding sweetpotatoes at low temperatures have been studied by many workers. Lauritzen (5) found that at temperatures below 9° C. (48.2° F.), Yellow Jersey sweetpotatoes were susceptible to infections which did not normally occur at higher temperatures. Kimbrough and Bell (4) described the effect of cold on the internal condition of sweetpotatoes. Albert et al. (1) reported similar injury from chilling. Lutz (6) showed that sweetpotatoes when subjected to a short period of chilling developed more decay when not cured than when cured. Whiteman and Wright (7) concluded that less injury and decay occurred at 55° and 60° than at 50° or 70°.

Materials and Methods

The sweetpotatoes used in the experiments reported here were the main commercial varieties—Big Stem Jersey, Yellow Jersey, Maryland Golden, Nancy Hall, Orange Little Stem and Porto Rico—grown in the nearby sweetpotato districts of Maryland, Virginia and New Jersey, each variety being obtained in the district where it is successfully grown commercially. These varieties represent both moist and dry types. They were dug in the morning and hauled in closed-panel delivery trucks to storage in the afternoon of the same or the following day. Every effort was made to obtain material from fields free of black rot at digging time, to avoid the complication of black rot development in the stored material. Before storage the sweetpotatoes of each variety were distributed equally among 27 half-bushel baskets. An effort was made to secure uniform distribution with respect to both size and

[1] Received November 17, 1952.

field location by distributing the potatoes from each field basket among all 27 storage baskets, with due regard to sizes. The baskets were individually weighed and placed immediately in the curing room at 85° F. with relative humidity near 85%. After ten days in the curing room the 27 baskets of each variety were weighed again and divided into three groups of nine baskets, one group being placed at 50°, one at 55°, and one at 60° storage. The temperatures were mechanically controlled so that fluctuations above or below the desired point were usually slight—not over one degree Fahrenheit. The main deviations from the desired temperatures were as follows: The temperature of the 50° room dropped to 30° for two days (Jan. 24–25, 1950). Again in 1950 it dropped to 45° for one day (Oct. 18), but only Orange Little Stem and Maryland Golden varieties were in storage at that time. Other variations of temperature in this room were slight and for short periods. Twice in 1949 the temperature of the 55° F. room dropped to 52° for two-day periods —once Nov. 7–8 and again Nov. 27–28. Otherwise the temperature of this room varied only slightly from that desired and for only very short periods. The 60° room was maintained all the time at 60° with only slight variations for short periods. Fans circulated the air in the storage rooms.

Three baskets of each variety at each temperature were removed from storage at each of three examination dates. All data given are averages for three years (1948, 1949, 1950). The percentages of sound potatoes, the weight losses in curing and storage, and the losses from rots

TABLE I

PERCENTAGES OF SWEETPOTATOES * OF SIX VARIETIES THAT WERE SOUND AFTER STORAGE FOR THREE, FIVE AND SEVEN MONTHS AT THREE TEMPERATURES

Duration and temperature (° F.) of storage	Big Stem Jersey	Yellow Jersey	Maryland Golden	Nancy Hall	Orange Little Stem	Porto Rico	All varieties
	%	%	%	%	%	%	%
3 months							
50	88.2	82.7	78.1	86.1	74.5	85.7	82.4
55	86.8	90.6	84.2	87.8	84.7	87.8	87.0
60	83.9	92.4	83.8	85.3	80.9	90.3	86.1
5 months							
50	59.2	25.0	55.7	52.1	18.4	78.9	49.3
55	82.0	86.9	78.8	80.6	79.7	85.6	82.3
60	84.8	90.1	82.2	80.3	77.2	88.0	83.8
7 months							
50	23.8	2.6	13.1	16.0	1.0	65.4	20.3
55	75.5	83.0	71.4	71.0	65.7	83.8	74.6
60	81.7	87.3	74.2	77.3	75.3	85.1	80.2
Variety average	74.7	70.8	69.1	70.7	61.9	83.4	...
	Varieties **		Storage conditions †			Variety × storage conditions ††	
L.S.D. 5%	12.0		4.7			16.3	
1%	17.0		6.3			21.9	

* Percentages based on original weights before curing—averages over 3 years.
** L.S.D.'s for testing variety averages.
† L.S.D.'s for testing all varieties average for the nine different storage conditions.
†† L.S.D.'s for testing the mean values given in the main body of the table.

were all calculated on the basis of the original weight of the potatoes. The percentages showing pithiness were based on samples of 45 externally sound potatoes (15 from each basket). The potatoes were sliced lengthwise for this inspection. The percentages that sprouted under good conditions were based on samples of nine potatoes, three from each basket. Sprouts emerging above the bedding sand were recorded separately from those not emerging. Two more tests, one to determine the amount and kind of discoloration of the flesh of freshly cut raw potatoes and the other the quality of the baked potatoes, were conducted, and the results were used in the final judgment of the response of sweetpotatoes to various storage conditions.

Results

Sound Potatoes. The externally sound sweetpotatoes remaining after losses from shrinkage and removal of decayed ones would generally be considered marketable regardless of their internal appearance or quality. An analysis of variance of these data was made to determine significant effects of variety, storage temperature and storage period on the percentages of sound sweetpotatoes. Since statistically significant differences were found for all three of these factors, pertinent data illustrating the differences are presented in Table I.

When all treatments are considered, Porto Rico kept significantly better than all other varieties except Big Stem Jersey. The high-carotene mutations Orange Little Stem and Maryland Golden did not keep as well as their respective parent stocks, Yellow Jersey and Big Stem Jersey, but because of variable responses in the different years these differences were not statistically significant, even though a difference was found at all storage temperatures and inspections.

From an inspection of all variety averages it is seen that the percentages of sound potatoes became less with each period of examination, as would be expected. In the analysis of variance the overall effect of storage temperatures as well as the interaction of storage temperature and inspection was highly significant. After five months of storage the percentage of potatoes stored at 50° F. that were sound was less than at 55° or 60°. This difference was even greater after seven months storage when there were fewer sound sweetpotatoes among those stored at 55° than among those at 60° (Table I).

From the standpoint of storage house management, the response of each variety to duration and temperature of storage is probably more important than the average for all varieties. After three months storage at 50° there was a tendency for the percentage of sound sweetpotatoes to be somewhat less than at 55° or 60°, but the difference was not significant. This difference was more pronounced for Little Stem Jersey and Orange Little Stem varieties than for the others.

After five months storage all varieties except Porto Rico had significantly smaller percentages of sound potatoes after storage at 50° F. than at 55° or 60°. There were appreciably fewer sound potatoes of even the Porto Rico variety from the 50° storage than from 55° or 60° (Table I). The difference between 55° and 60° storage was not significant for any of the varieties at this examination period.

For all varieties except Porto Rico there was a highly significant reduction in the percentage of sound potatoes after seven months storage at 50° F. over those stored at 55° or 60°. The difference for Porto Rico, significant at the 5% level only, again reflects the ability of this variety to withstand storage at 50° better than the other varieties. The differences in the percentage of sound

potatoes after seven months storage at 55° and at 60° were not significant for any variety, though the percentages were consistently larger for 60° storage. At 60° for seven months the percentage of sound potatoes ranged from 74 to 87% of the original weight for all varieties. On the other hand, at 50° for seven months the sound potatoes of all varieties except Porto Rico ranged from only one to 24%.

Loss Due to Decay. The percentage (by weight) of potatoes showing decay is recorded in Table II. Loss due to decay was much greater in some varieties than in others. It was in the main greater in all varieties after seven months of storage than after three or five months, as would be expected, and was much greater at 50° F. than at 55° or 60°. After five months storage the loss from decay at 60° was not consistently different from that at 55°, but after seven months it was consistently somewhat less at 60° than at 55°.

TABLE II

LOSSES DUE TO DECAY IN SIX VARIETIES OF SWEETPOTATOES STORED FOR THREE, FIVE AND SEVEN MONTHS AT THREE TEMPERATURES

Duration and temperature (° F.) of storage	Big Stem Jersey	Yellow Jersey	Maryland Golden	Nancy Hall	Orange Little Stem	Porto Rico	All varieties
	%	%	%	%	%	%	%
3 months							
50	3.8	7.9	12.6	6.6	16.1	6.1	8.9
55	4.8	0.0	6.2	4.6	4.6	4.4	4.1
60	8.9	0.2	7.7	8.3	10.2	3.2	6.4
5 months							
50	29.6	59.4	30.4	37.5	66.5	9.8	38.9
55	6.8	0.5	7.9	9.9	6.0	3.6	5.8
60	6.6	0.3	6.7	11.4	11.2	2.7	6.5
7 months							
50	59.2	68.7	66.6	66.8	75.9	18.9	59.4
55	10.4	1.8	12.7	17.1	15.9	3.5	10.2
60	7.0	0.8	10.8	12.1	9.3	2.7	7.1
Variety average	15.2	15.5	18.0	19.4	24.0	6.1	...

Weight Loss. The average weight losses during curing and storage for the 27 baskets of each variety is given in Table III. Maryland Golden and Big Stem Jersey showed greater loss in curing than Porto Rico and Nancy Hall. Appleman et al. (2) found that Maryland Golden lost 5.6% while being cured at 86° F. and 80 to 85% relative humidity, and 0.8% when cured at the same temperature at 100% humidity. Very little decay occurred during curing, and, therefore, practically all the weight loss was due to transpiration and respiration instead of decay. The weight loss in all varieties for each storage period was less at 60° than at 55° or 50°.

Internal Condition. Internal condition of apparently sound sweetpotatoes may be such as to impair greatly their food and market value. Pithiness, an internal condition, is characterized by whitish streaks and empty pockets in the flesh, and severely affected potatoes are light in weight and granular when cooked. Pithiness gives evidence of being a manifestation of senility. It may be confused with another breakdown characterized by more or less localized cavities in the flesh. This breakdown, which we call "cavity breakdown", is apparently

associated with chilling, especially when non-cured potatoes are involved.

The percentages of potatoes showing distinct pithiness were recorded for each variety at each inspection (Table IV). Pithiness was evident in some of the potatoes of the Orange Little Stem variety at the first inspection period (January), but it was not sufficiently developed at that time to impair the market value. By the middle of March up to 52 percent of the potatoes of this variety at 60° F. of the flesh of externally sound potatoes was found in some varieties. Porto Rico and Nancy Hall showed more discoloration than the Jersey types. In most cases the discoloration was associated with an unfavorable storage condition such as a temperature of 50°.

Sprout Production. The effect of storage conditions on the ability of sweetpotatoes to sprout at bedding time is of practical importance in propagation. The percentage of potatoes sprouting

TABLE III

WEIGHT LOSSES IN SIX VARIETIES OF SWEETPOTATOES DURING CURING AND AFTER STORAGE FOR THREE, FIVE AND SEVEN MONTHS AT THREE TEMPERATURES

Duration and temperature (°F.) of storage	Big Stem Jersey	Yellow Jersey	Maryland Golden	Nancy Hall	Orange Little Stem	Porto Rico	All varieties
	%	%	%	%	%	%	%
None (curing period)	3.7	3.4	3.8	2.8	3.3	2.8	...
3 months *							
50	4.3	6.0	5.5	4.5	6.1	5.4	5.3
55	4.7	6.1	5.8	4.8	7.4	5.0	5.6
60	3.5	4.0	4.7	3.6	5.6	3.7	4.2
5 months *							
50	7.5	12.2	10.1	7.6	11.8	8.5	9.6
55	7.5	9.2	9.5	6.7	11.0	8.0	8.7
60	4.9	6.2	7.3	5.5	8.3	6.5	6.5
7 months *							
50	13.3	25.3	16.5	14.4	19.8	12.9	17.1
55	10.4	11.8	12.1	9.1	15.1	9.9	11.4
60	7.6	8.5	11.2	7.8	12.1	9.4	9.4
Variety average (in storage)	7.1	9.9	9.2	7.1	10.8	7.7	...

* Loss during curing or from decay not included.

showed distinct pithiness, while the stock from which it mutated (Little Stem Jersey) showed none (Table IV). Maryland Golden, a golden fleshed mutant of the Big Stem Jersey variety, also showed considerable pithiness, but not so much as Orange Little Stem. Big Stem Jersey, from which the Maryland Golden variety was obtained as a sport, was not greatly affected with pithy breakdown, even after seven months storage.

A discoloration, either brown or green, also seems to give a good index of the effect of the storage treatments on the life activity of the sweetpotatoes used in these tests. The percentages of potatoes sprouting after having had the different storage treatments were recorded (Table V).

The total number of sprouts per potato paralleled so closely the percentage of potatoes sprouting that only the last is reported here (Table V). The data on the ability to sprout, in the main,

TABLE IV

PERCENTAGES OF SWEETPOTATOES OF SIX VARIETIES AFFECTED WITH PITHINESS AFTER STORAGE FOR THREE, FIVE AND SEVEN MONTHS AT THREE TEMPERATURES

Duration and temperature (° F.) of storage	Big Stem Jersey	Yellow Jersey	Maryland Golden	Nancy Hall	Orange Little Stem	Porto Rico
	%	%	%	%	%	%
3 months						
50	0.7	8.1	0.0	0.0	3.7	5.9
55	0.0	1.5	0.7	0.0	6.7	0.7
60	0.0	0.0	0.7	0.0	10.4	0.7
5 months						
50	0.0	22.0	6.0	3.0	53.3	18.1
55	0.0	8.1	14.1	0.7	54.1	0.2
60	0.0	0.0	20.7	0.7	51.8	0.7
7 months						
50	0.0	... *	14.5	3.7	... *	11.1
55	0.7	11.8	36.3	0.0	76.3	0.7
60	0.0	0.7	40.7	0.0	73.3	0.0

* Sample not available because of decay.

parallel other indicators used to determine optimum storage treatment. After three months storage most varieties of sweetpotatoes showed appreciably fewer potatoes sprouting at 50° than at 55° or 60° F. After five months storage the differences were more pronounced. After seven months storage at 50°, the ability to sprout was greatly impaired in all varieties. Earlier work on the effect of temperature on sprout production has been published (3).

Culinary Quality. The final test of a storage treatment is the acceptability of the product as food at the expiration of a storage period. A sample of each replicate was baked and tasted by three judges, and the color and texture of the

TABLE V

PERCENTAGES OF SWEETPOTATOES OF SIX VARIETIES THAT PRODUCED SPROUTS WHEN BEDDED AFTER STORAGE FOR THREE, FIVE AND SEVEN MONTHS AT THREE TEMPERATURES

Duration and temperature (° F.) of storage	Big Stem Jersey	Yellow Jersey	Maryland Golden	Nancy Hall	Orange Little Stem	Porto Rico
	%	%	%	%	%	%
3 months						
50	51.9	77.1	48.1	40.7	81.4	92.6
55	63.0	85.2	77.8	100.0	96.3	100.0
60	85.2	100.0	100.0	100.0	100.0	100.0
5 months						
50	37.0	40.7	22.2	36.3	38.9	77.8
55	70.4	77.8	39.9	100.0	92.6	100.0
60	85.2	85.2	88.9	100.0	100.0	100.0
7 months						
50	44.4	38.9	11.1	.... *	.... *	74.1
55	88.9	77.7	57.1	66.3	88.9	96.3
60	81.5	74.1	73.3	100.0	100.0	100.0

* Sample not available because of decay.

baked potatoes were recorded. The results of this test are not tabulated, but in general they follow the same trend as the other indicators; that is, those stored at 50° F. for five and seven months showed loss of color and impaired eating quality. Whenever brown or green discoloration was present in the raw potato it persisted after cooking and was associated with a disagreeable flavor. This cooking test was not so definite as some of the other tests, but it supplements them and helps to complete the picture.

Discussion

This work shows how sensitive sweetpotatoes are to after-harvest environment. When suitable curing and storage conditions are provided, the spoilage of certain varieties is remarkably low. On the other hand, when slightly unfavorable storage conditions prevail during long-time storage, spoilage may be very heavy. If sweetpotatoes are given the relatively warm storage they require they can be readily stored from harvest until planting time. Some varieties are still excellent food after seven months storage. Storage at 50° F. for three months shows little harmful effect and probably led to the erroneous conclusion from some of the earlier work that 50° is a suitable temperature for storing sweetpotatoes. However, the results of the present work indicate that long-time storage at 50° caused heavy spoilage in all varieties tested.

The data also emphasize the fact that the storage life is much shorter for some varieties than for others. It was found that the Orange Little Stem and Maryland Golden varieties should be consumed as soon after the middle of January as possible. When these varieties were held in storage longer they became light in weight, pithy and less desirable for food.

The wide divergence in response of the different varieties (moist and dry types) to the complexities of storage life is noteworthy. The various degrees of tolerance of these varieties toward adverse storage temperature are especially striking. The most widely grown variety, Porto Rico, showed more tolerance toward 50° F. storage than any other variety tested, while Yellow Jersey showed much spoilage at that temperature. When Yellow Jersey was stored at 55° or 60° it showed splendid keeping qualities.

The variation in the responses and tolerances of different varieties of sweetpotatoes to storage temperatures should be carefully considered in handling and marketing operations.

Summary and Conclusions

Six commercial varieties of sweetpotatoes were cured and then stored at 50°, 55° and 60° F. for three, five and seven months. The effects of these conditions on spoilage, weight loss, sprouting and pithiness were recorded. The amount of discoloration in raw potatoes and quality after cooking were evaluated and found to be similar to the other tests for quality at the different temperatures.

At the end of three months storage at 50°, 55° and 60° F., there were no significant differences in the percentage of sound potatoes.

After five months storage the percentage of sound potatoes of all variety averages at 50° F. was significantly less than at 55° or 60°.

After seven months storage this difference was even greater.

The data indicate that a moderate storage temperature of 55° to 60° F. is necessary for successful storage of all of the sweetpotato varieties tested, although there were marked varietal differences.

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Utilization Abstracts

Tannin from Waste Bark. Under this title, Bulletin 39 (31 pp., $2.50) of the Northeastern Wood Utilization Council (Box 1577, New Haven 6, Conn.) and published in 1952, consists of five articles dealing with tanning materials in general, the tanning industry of New England, the operation of a tannin extraction plant, potential tannin supplies from domestic barks and the methods of separating barks.

Cassia. Cassia flowers, used in perfumery, are gathered from the beginning of September to the end of November, or even into December if the temperature permits, from trees of *Acacia Farnesiana* Willd. cultivated for the purpose in southern France. The species is of Indian origin but was introduced into Europe about 1656. Each tree furnishes one to two pounds of flowers by its fourth or fifth year. In 1918 about 200,000 pounds of blossoms were treated in the perfume factories of Grasse; in 1949 only about 2,000 pounds. (Anon., *Am. Perf. & Ess. Oil Rev. 61(4): 280. 1953*).

Industrial Raw Materials of Plant Origin. I. The Division of Plant Exploration and Introduction, U. S. Department of Agriculture, has instituted a program " to investigate the needs of industry for plant-derived raw materials and to conduct market surveys for various commodities worthy of possible future development ". Formulation of the market surveys is being conducted by the State Engineering Experiment Station, Georgia Institute of Technology, Atlanta, Georgia, and is being published as a series of bulletins under the general title, " Industrial Raw Materials of Plant Origin ". The first of these appeared in 1952 under the sub-title " I. Recent Developments in Fixed Vegetable Fats and Oils ". It discusses the economic and technological developments regarding these products between January 1 and July 1, 1952, and the information presented was gathered from 18 journals dealing with the subject.

In addition to rather detailed production and cost data, as well as some notes on extraction processes and uses, of the better known oils (castor, linseed, oiticica, soybean, tung, babassu, coconut, cottonseed, olive, palm, peanut, pine, rapeseed, mustardseed), similar information is presented regarding so-called new oils, namely, those of black spruce, gokhru, grapeseed, kamala, mowrah, nim, pilchard, pisa, khakan, phulwar, queenroot, rice bran, safflower, sesame, sorghum and sunflower. (T. A. Wastler, P. M. Daugherty, H. H. Sineath, *Ga. Inst. Tech., State Engin. Exp. Sta., Bull. 11(1)*; also marked " *Bulletin No. 13* ". *1952*).

Industrial Raw Materials of Plant Origin. II. This second in a series of bulletins on economic and technological aspects in the utilization of particular plant products is devoted, in the words of its sub-title, to " II. Recent Developments in Vegetable Waxes, Gums, and Resins ", from January 1 to December 31, 1952. It is based on reports in 19 journals.

Pectin—*A Product of Citrus Waste*

The annual processing of citrus fruit wastes in the United States has reached two million tons. Forty thousand tons of pectin could be produced, compared with current production of three thousand tons. The physical and chemical properties of pectic substances are important botanically and industrially. Pectic substances aid in maintaining texture of fruits and vegetables and serve as jellying agents in preserves. The availability of increasing amounts of citrus wastes, combined with improvements in manufacturing techniques and new uses, promises expansion of industries concerned with pectin production and utilization.

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History of Citrus Culture

"Citrus" is the generic term for a great number of species of fruits grown in every country having suitable soils and climates. Webber and Batchelor (99) and Braverman (9) tell the interesting story of the spread of citrus culture throughout the world. The first written account of citrus fruit appears in an ancient Chinese book named "Yu Kung" or "Tribute of Yu" (an emperor who reigned from 2205 to 2197 B.C.). The book states that "The baskets were filled with woven ornamented silks. The bundles contained small oranges and pummeloes". Writings of Theophrastus, 300 B.C., mention citrons, probably the first genus known to the European civilization. Oranges and other citrus fruits were well known in other parts of the world at this time, yet only the citron was known to Europe up to about 1400 A.D. Sweet oranges were introduced into Europe and the countries of the Mediterranean region by the early part of the sixteenth century, and seeds of oranges, lemons and citrons were brought to Haiti in 1492 by Columbus. Florida and South Carolina had plantings of oranges in 1565, and considerable progress was made in citrus culture up to 1835. Oranges were introduced into California in 1769, and the first large orange grove was planted in 1804 at the San Gabriel Mission near Los Angeles. Oranges were grown at that time in Arizona, but commercial plantings were not made there until the early part of the twentieth century. Citrus culture in Texas and the Gulf States dates from about 1880.

Webber and Batchelor (99) present a complete account of the botany of citrus and the specific varieties that are now enjoyed by most of the world's population. The most widely grown and commercially important species are: sweet orange (*Citrus sinensis* (Linn.) Osbeck), grapefruit (*Citrus paradisi* Macfayden or *Citrus maxima* Merr.), lemon (*Citrus limon* (Linn.) Burmann, *Citrus limonia* Osbeck, or *Citrus limonum* Risso) and lime (*Citrus aurantifolia* (Christm.) Swingle).

The United States leads the world both in production and in processing of citrus fruits. Figure 1 presents processing and production data for the past 40 years.

Production increased tenfold and the quantity processed increased 3000-fold during the 40-year period from 1910 to the present. Citrus culls and processing

[1] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

wastes appear to be the most important source of pectin for present and future manufacturing.

The proportions vary slightly with the variety, but from 55 to 60% of bulk citrus fruits remain as peel, rag and seeds after processing (43). Current processing data show that about 2,000,000 tons of culls, peels and pulp were available in 1950–1951 in the United States. At present, 90% or more of this waste is dried and utilized as stock feed or as other less valuable material (69).

Table I presents the average chemical composition of grapefruit processing waste. If 60% of the pectin were extracted from this 2,000,000-ton mountain

TABLE I

ANALYSES OF GRAPEFRUIT CANNERY WASTE (78)

	Florida		California
	Peel	Rag	Peel and rag combined
	%	%	%
Total solids	16.71	15.60	22.02
Ash	0.74	0.75	0.70
Volatile oil	0.43	....	0.56
Acid, as citric	0.74	0.63	0.43
Crude fiber	1.71	1.44	2.00
Crude protein (N × 6.25)	1.13	1.06	1.63
Crude fat (ether extract)	0.28	0.16	0.23
Total sugar (as invert)	6.35	6.30	8.68
Pentosans	0.83	0.44	1.31
Pectin (calcium pectate)	3.10	3.56	3.93
Naringin	0.40	0.10	0.63

of waste, 40,000 tons of pectin would be obtained. Production in 1951 was only about 3,000 tons.

Occurrence of Pectin

Pectin [2], a jellying substance, is but one of the polysaccharides present in fruits and vegetables. Its discovery is credited to Vauquelin in 1790 (96), and Braconnot in 1825 (7) deesterified pectin to yield pectic acid. Since that time an impressive literature has accumulated to attest to the importance of pectic substances.

Kertesz in 1951 (34) published a comprehensive book containing more than 2,000 references on pectic substances. A review of historical interest (8) as well as more recent résumés (6, 23, 25, 31, 71), summarize the literature.

Research on pectic substances has been approached from two distinct points of view: (*a*) the relation of these substances to plant growth; (*b*) the properties that make them important as articles of commerce. Enzymatic and chemical changes during maturation that influence texture and keeping qualities of fruits and vegetables have been extensively investigated by botanists and plant biochemists. Food technologists and chemists have conducted research on those physical and chemical properties that show pectin to be unique as a jellying agent in the sugar-acid-water system of jellies and preserves.

The presence of pectic substances in the middle lamellae of plant cells was first suggested by Mangin (52). Development of pectin appears to occur at the time that the cell nuclei are beginning to divide (1). A pectic membrane divides the cell into two parts, and, as division continues, cellulosic membranes are formed with pectic material in the middle lamellae. Researches on the locus of pectic substances in plants, based upon histochemical staining reactions with ruthenium red, must be re-examined. Ruthenium red is now known to be unspecific for pectic substances (94). Recently, convincing evidence was presented that pectic substances occur in the middle lamellae of plant cells (5).

The extremely slow gain in our understanding of the biochemistry of pectin

[2] Nomenclature of the pectic substances follows that proposed by the Committee of Nomenclature, appointed by the Agricultural and Food Chemistry Division of the American Chemical Society in 1926, and is presented at the end of this article.

is due to the inexact knowledge of its composition and structure and of the morphology and taxonomy of plant cell structures. The electron microscope has recently been used to investigate the fine structure of cell walls and promises to furnish valuable information which could not be obtained by other methods (19, 63, 95, 102).

pectate gels *in vitro*. These same substances can promote disintegration of certain fruit tissue into individual cells or groups of cells no longer associated as a tissue. Whether or not calcium pectate is the chief cementing substance between cells awaits conclusive proof. Experiments on firming of tomatoes and apples by treatment with calcium salts

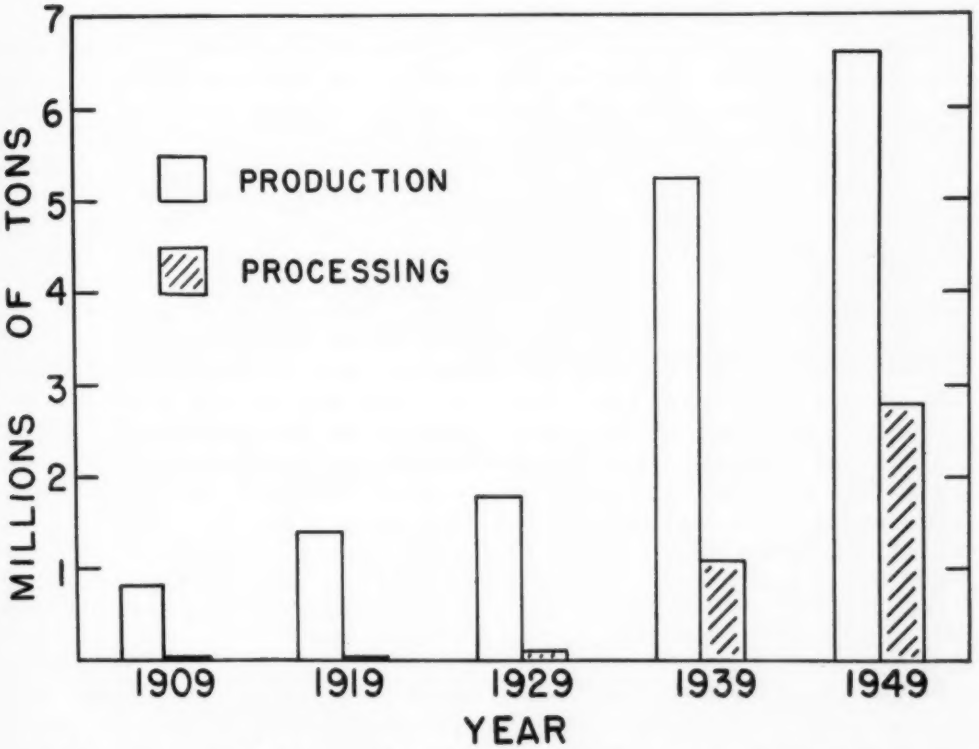

FIG. 1. Production and processing of the annual citrus crop of the United States increased 10- and 1,000-fold, respectively, over a 40-year period. (U. S. Bur. of Agr. Econ. Citrus fruits. 1909/10–1943/44, 1940/41–1946/47, 1946/47–1948/49, 1945–1949.)

The conviction held by some scientists that insoluble pectic substances e.g., calcium pectate, serve to cement individual cells together is largely based upon the behavior of pectic substances in vitro and on experiments with fruit tissues. Calcium complexing agents, such as oxalate, sodium polymetaphosphate and ethylene diamine tetra-acetic acid, as well as pectic enzymes, liquefy calcium

suggests that pectic substances are partly responsible for the texture of these fruits (42).

Pectic substances may serve to control the texture of some tissues, particularly of fruits. Pectin in unripe fruits has a high molecular weight, and as the fruit ripens, changes occur. These changes depend upon the type of tissue and the nature of the enzymes present.

One type of pectin degradation generally causes a softening of the tissue and proceeds to an extreme of complete disintegration.

Litle proof has been presented that pectic substances may or may not serve as reserve carbohydrates that can be metabolized by the cell. They seem to be deposited as part of the framework structure of the tissue, and in some cases the total quantity changes during ripening.

No progress has been reported on the mechanism of the synthesis and nonhydrolytic breakdown of the pectic substances in plants. Elucidation of this mechanism may answer many questions asked by plant physiologists and others concerning the texture changes that occur during maturation, storage and processing of fruits and vegetables.

Constitution and Chemical Properties

The discovery of pectin dates back 162 years, but its main constituents were discovered rather recently. Von Fellenberg in 1914 (18) showed that methyl alcohol is a part of the pectin molecule, and both Saurez and Ehrlich identified galacturonic acid as its main constituent in 1917 (16, 85). Other early workers (65) postulated that pectin is a ring compound containing arabinose, galactose and four galacturonic acid units esterified with methyl alcohol.

Smolenski (90) deduced that the galacturonide units were polymerized in a chain similar to the glucose units in starch and cellulose. Acceptance of his ideas were slow because of the inaccessibility of his original publications, although the physical properties of pectin supported the idea of a linear structure. Methanolysis of pectin to yield a compound containing at least 12 galacturonide units furnished the first chemical evidence indicating a linear structure (61). Subsequent work has shown that the product consists of 30 or more anhydrogalacturonide methyl ester residues united in chains (29). Methylation studies proved that the galacturonic acid units are α-linked through positions 1:4 (24). Exhaustive methylation of undegraded pectin has not been accomplished. However, even from the methylated derivatives obtained from the polymethyl ester, no tri-methyl galacturonic acid end group was reported among the products of hydrolysis (24, 25).

Pectin has a relatively complex structure, as compared with cellulose and starch. The structure of pectic acid is compared with that of cellulose in Figure 2. Although they are composed chiefly of anhydrogalacturonic acid residues united in chains, pectic substances are usually quite heterogeneous. For example, pectin is composed of polygalacturonide chains having a wide variety of molecular weights. Some of the carboxyl groups are esterified with methyl alcohol, some are neutralized with cations, and some are free acids. In addition to the heterogeneity with regard to molecular weights and methoxyl contents, pectins vary in the distribution of ester groups along the chains.

The question whether or not pectin is a pure polyuronide has been the subject of conflicting studies. It is known that degraded pectic acids from several sources are the same (25). In addition there is evidence that araban and galactan present in some crude pectin preparations cannot arise directly from polygalacturonides. Araban is a branched α-arabofuranoside, galactan a β-galactopyranoside. The course of synthesis of araban, galactan and pectic acid must, therefore, proceed at the monose-unit level. It has been reported that pectic substances are purified by successive precipitations in dilute ethanol (21). Examination of data presented, however, shows that the treated material contained only 86% anhydrouronic acid instead of the 96% required by the assumption that pectin is an esterified polyuronide.

Arabinose and galactose have repeatedly been isolated from the products of hydrolysis of pectin preparations, and these findings have been further confirmed with pectin rigorously purified purified passion fruit and citrus pectic acids, respectively (53, 57). Pectin from pumpkin, pomelo, guava and country pears purified by precipitation with dilute alcohol were nearly identical in

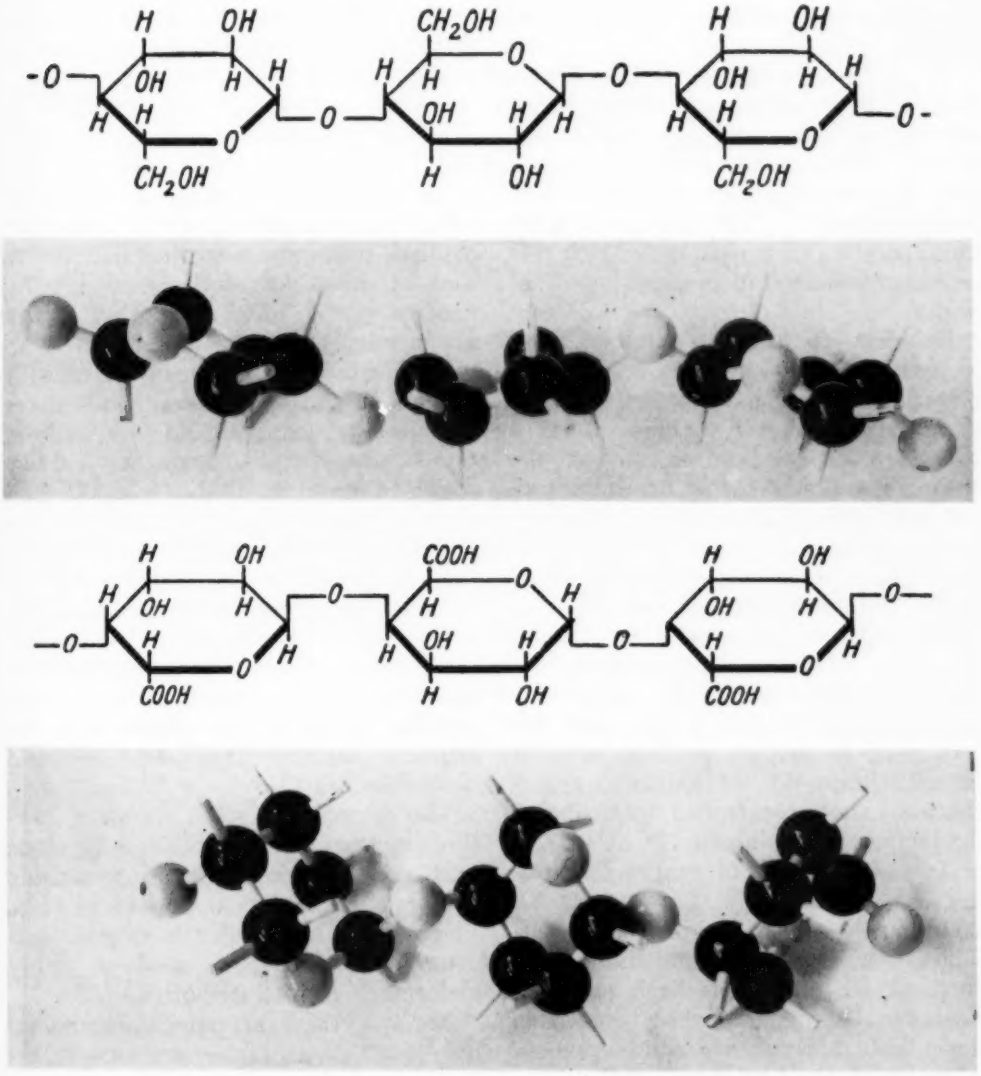

FIG. 2. The structural formula and model of cellulose showing its two-fold screw symmetry (above); and model of pectic acid with its three-fold screw symmetry (below).

by such methods as precipitation by polyvalent cations and repeated alcohol precipitations of pectic acid. L-Sorbose, D-galactose, L-rhamnose, L-arabinose and D-galactose have been isolated from composition but were not pure polyuronides (79).

The kinetics of the removal of the so-called "ballast" of apple pectin suggested that a substantial part is attached

to the polyuronide chain by covalent linkages and that part is held by hydrogen bonds (92). The exact composition of the undegraded chains of pectic substances cannot be considered as settled (25), and non-uronide sugars may be integral constituents of undegraded pectins.

The presence of acetyl groups in pectins from various sources has been reported by many investigators (17, 22, 64, 75). Apple pectin contains no acetyl; that of citrus has 0.3%, and sugar beet pectin has 4.5%. The amount of combined acetic acid can vary with the source and method of preparation of the pectin.

Henglein et al. (20) claimed that phosphoric acid, as esters or in ionic linkage with pectin, plays a part in bridging between the chains.

Much work has been reported on the chemical and enzymatic breakdown of isolated pectin, with respect to both de-esterification and glycosidic cleavage of the chains. Acid de-esterification of pectin has an energy of activation of 17,000 cal., and glycosidolysis 30,000; therefore, at lower temperatures, de-esterification proceeds more rapidly than depolymerization (92). This fact has been used to prepare pectinic acids of different properties. Alkaline de-esterification can be controlled by reducing the temperature to about 15° C. (59) to make pectinic acids of commercial usefulness.

Ordinarily glycoside groups are considered stable to alkali, and the lability of pectin in alkali has been puzzling. Recently the suggestion has been made that alkali catalyzes a β-elimination reaction, as shown in Figure 3 (98).

Pectic acid has been found stable to dilute alkali and unstable in alkaline methyl alcohol solution, which is in agreement with this suggestion. Additional work must be done to prove β-elimination to be the mechanism responsible for the alkaline degradation of pectin chains.

Whereas chemical de-esterification of pectin takes place at random, enzymatic de-esterification catalyzed by pectinesterase is specific. Glycosidic hydrolysis of pectin occurs with the enzyme polygalacturonase. The action of enzymes on pectic substances will be discussed later.

Pectin is degraded by a number of oxidizing agents: hydrogen peroxide (13), endiols or ascorbic acid and peroxide (83), auto-oxidizing ascorbic acid (33), and periodate (37). Periodate oxidizes pectin in a manner similar to that of other polysaccharides, but the mechanism by which the other agents act is unknown.

Although pectin can be esterified with methyl alcohol-hydrochloric acid, silver oxide-methyl iodide or dimethyl sulfate, the only method that seems to yield undegraded products is the reaction with diazomethane at low temperature (97). Epoxides have been used to esterify pectin and pectic acid, but there is some evidence that cross-bonding occurs (14).

The amine salts of pectic substances are readily prepared by direct neutralization with the free base (93), and transesterification with pectin and dry aliphatic amines takes place to yield n-alkylamides (11).

The secondary alcohol groups of pectin have been nitrated with fuming nitric acid (21) and acylated after swelling in formamide with acid anhydrides (12). These esters are soluble in organic solvents and have been used to study molecular weight distributions (68).

Methyl ethers of polygalacturonides have been prepared for structure determinations (45).

The presence of 2,3-dimethyl galacturonic acid in the furanose configuration was explained as a transformation of the expected 2,3-dimethyl pyranose into the furanose form under certain reaction conditions. No 2,3,4-trimethyl galactu-

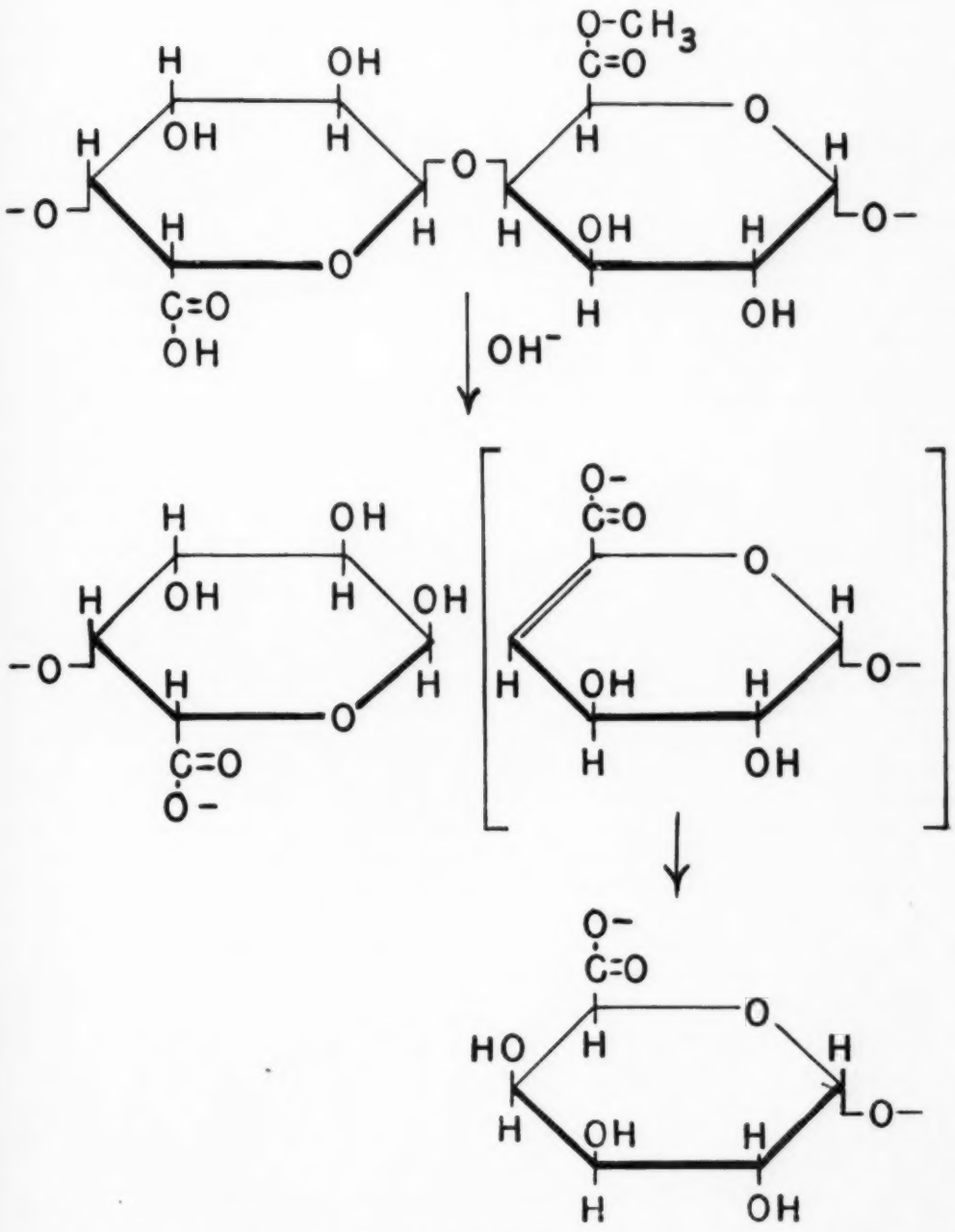

FIG. 3. Tentative scheme of a possible β-elimination reaction applied to explain the alkali lability of the glycosidic linkages in pectin.

ronic acid was reported among the hydrolysis products of the methylated derivatives to show possible branching or give an indication of the chain lengths.

There is probably but one free reducing group per molecule of pectin, as with other polysaccharides (starch and cellulose). This possibility, along with the alkali lability of pectin, makes reliable interpretations of its reducing properties difficult. Isbell's method (27), using radioactive cyanide to measure potential aldehyde groups in polysaccharides, may aid in explaining some of the reducing properties of pectic substances.

Although much has been done to elucidate the chemistry of this reactive and complex group of polygalacturonides, the mechanism of biological synthesis, alkaline and oxidative degradation, and exact composition are still unknown.

Physical Properties

As a polymer of galacturonic acid, pectin has many physical properties not associated with other polysaccharides commonly found in fruit tissue. Its unique properties are due primarily to the carboxyl group. When pectin is completely esterified, its acid, viscosity and gel behavior are far different from those encountered in commercial or natural pectin. The fully esterified material has no free acid, but if only a few carboxyl groups are available on each pectin molecule, the ionization constant is near that of galacturonic acid. As more groups become available, each affects he other so that the ionization constant is depressed in much the same way as the second ionization constant of tartaric acid. In the case of pectin the pK values (log of the reciprocal of the ionization constant) increase from 3.42 to 4.38 in going from the almost completely esterified molecule to pectic acid (76). The pK value varies not only with the number of free carboxyl groups per molecule but also with the concentration. So far, recent theories of polyelectrolytes have not been applied to pectin, but it appears that the derivation for rod-like polymeric acids will be applicable (77).

The carboxyl groups influence the viscosity of pectin solutions. Solutions of fully esterified pectin do not change appreciably in viscosity with change in pH, but when they contain lower-ester pectins the viscosity becomes markedly pH dependent. Apparently, as the carboxyl groups ionize with increase in pH, electrostatic repulsion lengthens the chain, causing more intermolecular interactions and an increase in viscosity. If salt is added to shield the carboxyl groups, the electrostatic effects are reduced and the viscosity drops to about the same value as measured in the presence of excess acid. With low-methoxyl pectin (less than 50% esterified) and pectic acid, association between molecules becomes appreciable below pH 4 and precipitation occurs.

The carboxyl groups also contribute to the gel characteristics of pectin. Above pH 3.5, where pectin is 40% ionized, it is difficult to prepare sugar jellies. Again, this difficulty appears to be due to the repulsion between the negatively charged groups. When the pH is reduced to lower values, jellies can be easily formed. The sugar or similar hydrophilic substance is required to reduce the solvation of the pectin molecule. When these two conditions are met (low pH and high sugar content), the pectin molecules associate probably through hydrogen bonds involving the carboxyl alcohol groups.

Gelling pectins fall into three classes: rapid set, slow set and low-methoxyl. They are to some extent dependent upon the methoxyl content or degree of esterification (DE). The classes are arbitrary, and the boundary lines be-

tween them are not sharp. Generally, a pectin with a DE of 70% or above will form a jelly more rapidly or at a higher temperature than one with a DE between 50 and 70%. Pectins with DE's below 50% are not commonly used to make high-solids gels, but they cause rapid gelation. This distinction among pectins seems to be related to their solubility (32). Fully esterified pectins are not so soluble in the gelling medium as the ordinary pectins, while the low-methoxyl pectins tend to precipitate so rapidly in acid media as to form irregular gels.

Another factor controlling the viscosity, gel, film and fiber properties of pectin is its molecular weight. Although pectin is a high polymer, its molecular weigh is much below that for cellulose or starch. The number average molecular weight is between 30 and 70 thousand for citrus pectin; the weight average is 1.5 to 2 as great (68). The limiting viscosity number of citrus pectin solutions ranges from 200 to 1,000 ml./gm. These are much higher values than those reported for alkaline amylose or cellulose solutions.

The reason for the large contribution that pectin makes to the viscosity of its solutions apparently lies in the structure of the molecule. X-ray patterns have been interpreted to indicate that the unit cell has a length of 13.2 Å, corresponding to three galacturonide units (70). Each galacturonide has a projected length along the chain of 4.3 Å. This shortened projection, compared to the 5.1 Å for glucose units in cellulose, means that the galacturonides lie along the chain in a zig-zag fashion. When models are constructed corresponding to this interpretation of the X-ray diagram, it is learned that there is restricted rotation of the groups around the glycoside group (70). Many of the configurations available to cellulose molecules are thus denied pectin molecules, and the extended configurations are more predominant. At the same degree of polymerization and concentration, pectin solutions would be more viscous than cellulose solutions.

The molecular weight and chance for hydrogen bonding permit formation of pectinate films and fibers. They have tensile strengths comparable to those made with cellulose but are more brittle (86). Pectin can be cross linked with formaldehyde and other agents, so that this disadvantage can be overcome (15).

Many other physical properties have been studied, but they are somewhat specialized. In addition to titration behavior, electrophoretic mobilities show that pectin de-esterified in solution with acid or alkali yields low-methoxyl derivatives that are relatively homogeneous with respect to the distribution of methoxyl groups. On the other hand, pectinesterase produces a wide variety of de-esterified pectins. Apparently, the enzyme requires a free carboxyl to begin its action and then proceeds along the molecule. As a result, enzymatically de-esterified pectins contain molecules ranging from pectic acid up to the original high-methoxyl pectin. Even those molecules which are partly de-esterified may have part of the chain completely free of ester groups, while the remainder has an ester distribution like that of the original molecule. These differences are apparent in both the acid and electrophoretic behavior because at the same methoxyl content the enzyme-de-esterified samples are weaker acids than the acid- or alkali-treated ones. The pectic-acid portions of galacturonide chains have a very low calcium requirement for the preparation of low-solids gels; it is, therefore, more difficult to prepare a uniform and acceptable gel with an enzymatically partially de-esterified pectin than with alkali- or acid-prepared material.

The properties of pectin gels are important to the utilization of pectin in

jellies and other food products. Bonding between pectin molecules probably involves hydrogen bridges between free carboxyl groups and possibly between carboxyl and alcohol groups. The rapid setting of high-ester pectins suggests that other attractive forces must also play significant roles. For example, when pectin gels are very slightly compressed, the displacements appear to be a linear function of the load. If the stress is immediately removed, the gel recovers to its original dimensions. If the displacements are large, then they are no longer a linear function of the load. Instead, the stress-strain curve becomes parabolic in shape. Also, if the load is applied for a short period of time before removal, the gel does not recover. Gelatin gels act the same way but do not show such rapid stress relaxation.

Calcium pectinate gels are also similar, but, because cross bonding is due to electrovalent bonds, small deformations cause rupture and realignment of the bonds so that recovery after deformation is not so complete (91). Relaxation is not so rapid with these gels, but the rigidities are much more dependent upon temperature changes. The temperature dependence of the calcium pectinate gels reflects changes in the number of cross bonds as the solubility of calcium pectinate increases with rise in temperature. In the high-solids gels other attractive forces must account for the small change in strength as the temperature is increased from 0 to 60° C.

Pectic Enzymes

Hydrolytic cleavage of methyl ester and glycosidic bonds in pectic substances is catalyzed by the enzymes pectinesterase (or pectase) and polygalacturonase (or pectinase), respectively. Recently other glycoside-splitting enzymes have been characterized. Interest in these enzymes has been stimulated because of their essential roles in plant metabolism and their industrial usefulness in juice clarification. Nomenclature [3] of the enzymes has been as unstable as that of their substrates, the pectic substances. There are several thorough reviews of the subject of pectic enzymes in the recent literature (35, 36, 41, 72).

Pectinesterase (PE) attacks the methyl ester groups of pectinic acid, converting it ultimately to pectic acid. The enzyme is widely distributed in nature and has been reported in alfalfa (38), tomatoes (55), citrus (48, 66), fungi (55) and in leaves (26).

Although PE occurs throughout the plant, in most cases it is found to be associated with the insoluble portions of the tissue. Citrus pectinesterase requires both the addition of salt and careful pH adjustment to be extractable from the insoluble solids of the fruit (48). Use of 0.25 *M* sodium chloride at pH 7 to 8 for about an hour will probably extract most of the activity from plant tissue.

The method of PE assay recommended (after a suitable enzyme preparation is obtained) is that of determining increase in carboxyl groups liberated from pectinic acids at constant pH. Cations, the alkaline de-esterification of pectin, pH and temperature all affect the activity, and conditions for assays are rigorously specified. For citrus PE, the conditions defined by MacDonnell et al. (48) are pH 7.5, temperature 30° C., sodium chloride 0.15 *M* and 0.5% citrus pectin (methoxyl about 10%). The activity is conveniently expressed as milliequivalents of bonds hydrolyzed per minute at optimum conditions.

PE hydrolyzes the methyl ester groups of pectin, ultimately converting it to pectic acid. The fact that contiguous methyl ester groups are hydrolyzed along

[3] The nomenclature used here is that proposed by the Committee on Pectin Nomenclature of the American Chemical Society and presented later with that for the pectic substances.

the pectin chain suggests that PE remains combined with the pectin molecule until most of the ester groups are hydrolyzed (41). No hydrolysis of methyl galacturonate or methyl galacturonide methyl ester is observed. The effect of cations on increasing PE activity and the shift in pH optimum in the presence of cations have been explained by Lineweaver and Ballou (39). Cations do not activate PE as such but may prevent its inhibition by the carboxyl groups of pectin and pectic acid in acid solutions. In alkaline solution the carboxyl groups are ionized and do not combine with the electrostatically charged enzyme (39).

It is now clear that PE, when pure, is highly specific (47). PE has a fairly low temperature coefficient, similar to that of other esterases. It is fairly heat-sensitive and labile in acid solution. PE is not readily inactivated by chemical means; however, it is reported to be inactivated by detergents (55).

Polygalacturonase (PG) from fungal origin acts on the de-esterified portions of polygalacturonide chains, breaking the 1,4-glycosidic linkages and producing materials of low molecular weight. While PG does not attack completely esterified pectins, the combined action of PE makes them susceptible to PG attack (28). It is evident that great confusion regarding PG action would arise if the enzyme preparation contained PE.

PG occurs in many bacteria, fungi, snails, yeast (46, 73) and higher plants (56, 62). The properties of PG from various sources differ. Additional work is needed, since most of the enzyme studies reported have been done with fungal PG.

The end products of the action of fungal PG on pectic acid have not been identified completely (30, 81, 82). The inference from the rate of hydrolysis of PG on pectic acid is that the main product at 50% hydrolysis is digalacturonic acid. After prolonged action galacturonic acid is produced. Whether or not galacturonic acid is produced from digalacturonide by a second enzyme in the purified PG preparation is not known. No experimental evidence in support of the latter theory has been presented. Mono-, di- and trigalacturonic acids in equal molar ratios have been reported as the end products of the hydrolysis of pectic acid by yeast polygalacturonase (46, 74).

Action of fungal PG on pectic acid-producing materials of low molecular weight naturally results in an increase in reducing groups, decrease in viscosity, decrease in precipitability with calcium or alcohol, and decrease of the optical rotation. The assay method preferred by most is that based upon an increase in reducing groups. Jansen and MacDonnell modified the Willstätter-Schudel hypoidite method, and the reaction conditions recommended are 0.5% pectic acid adjusted to pH 4.0 at 25° C. (28). The results are expressed as millimoles of reducing groups liberated per minute. The hydrolysis of pectic acid by PG was zero order to 50% hydrolysis.

Viscosity methods have been used to obtain relative activities of PG preparations. The expression of activity is more complex than the expression using reducing values.

Purified fungal PG seems to be very specific (40), but accurate enzymatic activities can be obtained only with purified pectic acid. Crude enzymes and substrates produce arabinose and galactose from non-uronide moieties and thus confuse the picture (30, 81). Most PG purification procedures are based upon the greater stability of PG over PE in acid solution at room temperature. PE is inactivated by absorption and elution from acidic ion exchange materials (54). Fungal PG was only 50 to 70% inactivated at pH 0.6, for 20 minutes at 23° C., while PE was inactivated (40).

Evidence has been presented that pectic enzymes besides PE and PG exist. These macerating enzymes have actions different from PG and may in fact be the macerating enzymes found in certain plant tissue. The inferential and direct evidence is discussed in recent reviews cited earlier (35, 41).

Roboz et al. (84) reported the presence of an enzyme produced by *Neurospora crassa,* which hydrolyzes the glycosidic linkages of pectin or pectic acid but does not produce galacturonic acid. Wood (101) described an enzyme, produced by *Bacterium aroideae,* which attacks pectin, rapidly lowering the viscosity with little increase in reducing power, and has a pH optimum near 8.5. Seegmiller and Jansen (87), using a commercial product, partially purified an enzyme which can hydrolyze pectin (in preference to pectic acid) to products of low molecular weight. Galacturonic acid or methyl galacturonate was not present among the hydrolysis products. The pH optimum for their preparation, tentatively named polymethylgalacturonase (PMG), was near 6, and its action results in the cleavage of about 26% of the glycosidic bonds of pectin.

Before questions regarding specificity and mode of action can be answered, it will be necessary to obtain enzymes and substrates of higher purity and known composition. If it is established that non-uronide sugars exist in the polygalacturonide chains as part of the pectin molecule, some of the differences in the activities of the glycoside-splitting enzymes may be explained.

Commercially, pectic enzymes are used to clarify fruit juices and aid filtration. They are also used to de-esterify pectin to low-methoxyl pectin, and in the manufacture of citrus pulp and molasses. The pectic enzymes are of widespread occurrence, and their control in the fruit processing industry is important. Recently, increased interest has developed because pectic substances may control the texture of fruits and some vegetables during growth, processing and storage.

Information is needed on the preparation, purification and properties of enzymes of higher plants. Additional experiments should be conducted on the purification and determination of the structure of their substrates, the pectic substances.

Manufacture of Citrus Pectin

The process for manufacturing pectin should not be considered as a process for disposal of citrus wastes. It should be regarded as a special enterprise to obtain additional values which would otherwise be lost if the waste were processed only into low-cost feed or soil conditioner.

Citrus peel is made up of flavedo, the thin external layer of pigmented and oil-containing cells, and albedo, the spongy layer (rich in pectin) that makes up the bulk of the tissue.

The most suitable sources of citrus wastes for the manufacture of pectin are the peels of oranges, grapefruit and lemons. The objective of most manufacturers is to prepare pectin of high jellying power, and the yield of product is of secondary importance, since most citrus pectin manufacturers known to the authors use fresh waste, although use of dried waste prepared under carefully controlled conditions has been considered (88).

Pectin is sold on the basis of its jelly grade, defined as the number of pounds of sugar that one pound of pectin will cause to set in an acceptable jelly prepared by a standard procedure. The manufacturing procedures vary somewhat but in general consist of the following steps: preparation of materials, extraction of the pectin, clarification of the pectin-containing solution, concentration, isolation, drying, grinding and packaging.

Usually the peel is finely sliced and

subjected to water washing. This serves to remove sugars, some glycosides such as hesperidin and naringin, coloring matter and other soluble constituents. Some pectin is usually lost in this step.

The leached peel is now ready for extraction. The pectic enzymes have not been inactivated; therefore, the peel must be extracted as soon as possible. The leached peel is extracted at about pH 2.0 with hydrochloric or sulfuric acid at temperatures from 80 to 100° C. and for periods of 30 minutes to an hour or more. Such treatment serves to disintegrate the material, break hydrogen bonds and calcium or other salt linkages, and speed diffusion of the large pectin molecules into solution. This operation is somewhat expensive because of the need for corrosion-resistant equipment capable of handling hot dilute mineral acids. The hot pectin solution is separated from the bulk of the insoluble matter by roll-presses, vibrating screens or hydraulic presses. The pectin solution must then be further clarified.

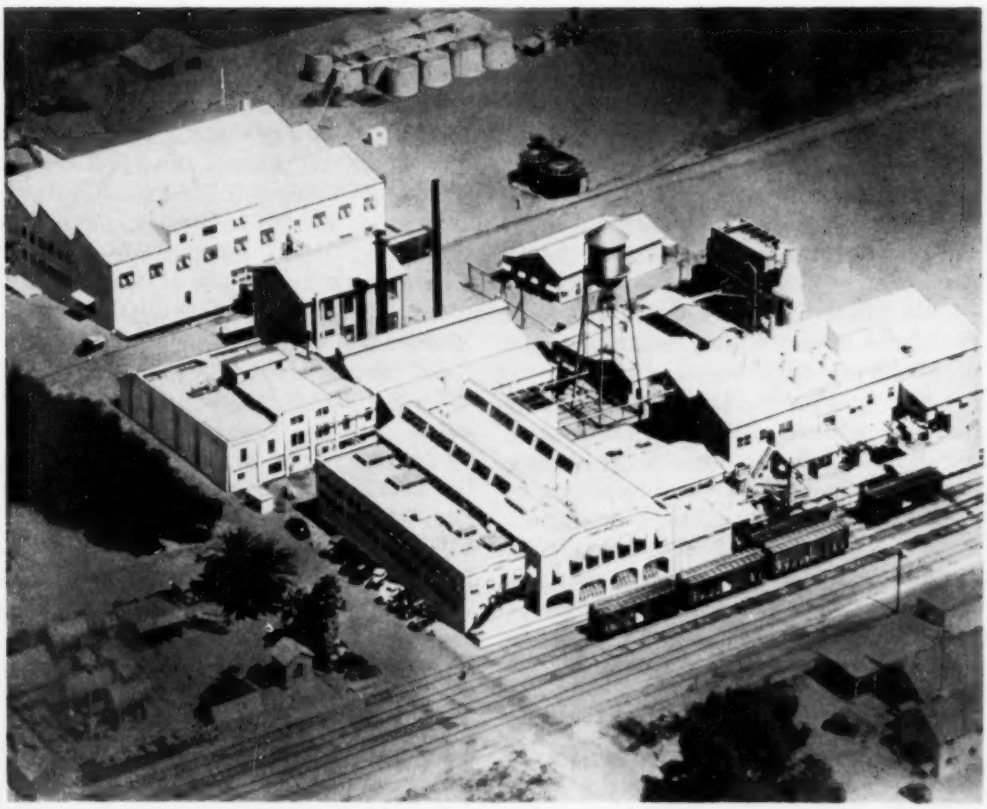

FIG. 4. A modern citrus byproducts plant provides facilities for a wide variety of activities. Operations (left to right) include juicing, isolation of citric acid, processing of juice into concentrate, chemical research and control, and at right, the pectin plant.

Clarification of the turbid solution may be accomplished by centrifugation or filtration on screens or cloths of plate-and-frame filters. Because of the high viscosity of pectin solutions, the concentration of the extract must be kept at 0.5% or below and the temperature at 60 to 70° C. From 0.5 to 1% of diatomaceous-silica filter-aid is required for this expensive operation.

The clarified pectin solution is cooled and concentrated by suitable means. Concentration *in vacuo* is necessary if evaporative methods are to be considered because the jelly grade of pectin decreases rapidly if the solution is boiled. Concentration in vacuum pans to about three or four percent pectin can be effected before the solution becomes too thick. A method of concentration involving metallic ion precipitation is also used (100). Aluminum sulfate is added to the pectin solution, followed by sodium carbonate or ammonia. When the pH reaches 4.2 to 4.4, the negatively charged pectin flocculates with the positively charged aluminum hydrosol, and the aluminum-pectin complex floats to the surface. Usually a small amount of copper sulfate is added which toughens the flocculated material, making the pressing operation easier.

Some pectin is sold in liquid form; most is marketed as dried powdered material. Several methods of producing dry pectin from the concentrated material have been used. Concentrated pectin solutions have been spray-dried, drum-dried and precipitated with alcohol, pressed and air dried. The spray- and drum-drying methods are perhaps the most economical. They require the concentrated pectin solutions to be very pure; otherwise salts, mineral acids, sugars and other impurities will contaminate the finished pectin. Precipitation of the concentrated pectin solution with 60% alcohol (ethanol or 2-propanol) is used commercially. The pectin flocculates and is pressed, treated with a fresh portion of alcohol, pressed again and dried. This procedure requires the use of a very efficient solvent recovery system, since about 60 pounds of alcohol are required in the preparation of one pound of dry pectin. The alcohol-precipitation method yields high-quality pectin, white in color and free from bitter flavors. Dry pectin is obtained from the aluminum-pectin complex by washing out the metal salts with acidified alcohol. The aluminum-pectin complex is first pressed to about 10 or 20% solids and then extruded into acidified alcohol. The metal salts are dissolved in the acidified alcohol and the pectin is given a final wash with alcohol to remove excess acid; it is then dried at about 70° C. The pectin produced in this process is of good quality and purity. About the same amount of alcohol is required in this as in the other process.

Various methods have been used to prepare citrus pectin commercially, but they consist essentially of modifications of one or more of the steps outlined above.

Low-methoxyl pectin shows promise as an article of commerce, since it can be used in a wide variety of applications. A process for the production of low-methoxyl pectin from orange peels without use of organic solvents has been proposed (67). The process is based upon the fact that low-methoxyl pectins can be precipitated from aqueous solution with dilute acid (60). The precipitated pectin is pressed, washed with water, partially dried and partially neutralized with sodium bicarbonate.

The future possibilities of increased production of pectic substances depend upon refinements in technique which, combined with other economic factors, will result in lowering the production costs and permit their expanded utilization.

Uses

Pectin causes gelation in the sugar-acid-water-pectin system of jellies and jams. The bulk of the pectin produced is used commercially and in the home for the production and preparation of fruit spreads, including jellies, jam, preserves and the like. The composition of these products is rather exactly defined by the pure food laws in the United States. For

example, jellies must contain not less than 65% soluble solids and have a ratio of at least 45 pounds of fruit juice to 55 pounds of added sugar in the original batch. For details of the preparation of these and other pectin-containing food products, the reader is referred to Jam Manufacture (80), the Preservers Handbook (10) and other technological books on this subject.

Pectin has been widely used in a variety of confections. It has been found to be useful as a stabilizer for salad dressing, ice cream and pharmaceutical pastes (51). Pectin compares favorably with gum tragacanth, karaya and acacia as an emulsifying agent (44). Research has indicated that pectin, suitably prepared, may prove to be useful as a blood plasma substitute and as a blood agglutinant in the treatment of certain internal hemorrhages, and pectate has been widely used in the treatment of diarrhea.

In addition to the food, pharmaceutical and medicinal uses for pectin, new products have been developed which may find wide application. For example, low-methoxyl pectin produced by carefully controlled de-esterification can be used in the preparation of canned jelled fruit cocktail, milk puddings and low-solids fruit gels (49, 59). Practical application of films made of pectinate to coat candied fruits, meats, frozen foods and oily nuts has been described (49, 50). The film can be applied by dip-coating and is edible. A dessert can be prepared with low-methoxyl pectin, milk powder, flavoring, sugar and cold water (89). It is ready to eat in seven minutes. Commercial sodium pectate of high molecular weight shows promise as a coating agent in the same way as low-methoxyl pectin. A commercial enzymatically de-esterified high-molecular-weight sodium pectate has served experimentally as a substitute for agar in the preparation of a gel culture medium for the growth of bacteria (58) and can be used as a creaming agent for rubber latex and for hardening steel (4).

Pectin is the only permissible jellying agent for jellies, jams and preserves. The utility of pectic substances in other fields has already been demonstrated, and the reason for their slow acceptance is their high cost compared with the substances currently used. A promising future can be predicted for special pectin products if cheaper methods for their production can be developed.

Nomenclature of the Pectic Substances and Pectic Enzymes (2, 3)

" Pectic substances " is a group designation for those complex colloidal carbohydrate derivatives which occur in, or are prepared from, plants and contain a large proportion of anhydrogalacturonic acid units which are thought to exist in a chain-like combination. The carboxyl groups of polygalacturonic acids can be partly esterified by methyl groups and partly or completely neutralized by one or more bases.

" Protopectin " is applied to the water-insoluble parent pectic substance which occurs in plants and which, upon restricted hydrolysis, yields pectin or pectinic acids.

" Pectinic acids " is used to designate colloidal polygalacturonic acids containing more than a negligible proportion of methyl ester groups. Pectinic acids, under suitable conditions, are capable of forming gels with sugar and acid, or, if suitably low in methoxyl content, with certain ions. The salts of pectinic acids are either normal or acid pectinates.

" Pectin " (or " pectins ") designates those water-soluble pectinic acids of varying methyl ester content and degree of neutralization which are capable of forming gels with sugar and acid under suitable conditions.

" Pectic acid " is applied to pectic substances composed mostly of colloidal polygalacturonic acids and essentially

free from methyl ester groups. The salts of pectic acids are either normal or acid pectates.

"Protopectinase" is used to describe the enzyme that converts protopectin into a soluble product. It has also been called "pectosinase" and "propectinase".

"Pectinesterase" (PE), or "pectin-methylesterase", describes the enzyme that catalyzes the hydrolysis of the ester bonds of pectic substances to yield methanol and pectic acid. The name "pectase" does not indicate the nature of the enzyme action and has given way to the more specific names "pectinesterase" (PE) and "pectin-methylesterase".

"Polygalacturonase" (PG), or "pectin-polygalacturonase", is used to describe the enzyme that catalyzes the hydrolysis of glycosidic bonds between de-esterified galacturonide residues in pectic substances. "Pectinase" is frequently used to designate the glycosidase and also for pectic enzyme mixtures.

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Utilization Abstracts

Hemlock. The name "hemlock" is applied to two extremely different groups of plants. One of them, constituting the genus *Tsuga*, consists of ten or so species of forest trees in temperate North America, Japan, China and the Himalayas; two of them (*Tsuga canadensis* and *T. caroliniana*) are native to the eastern United States, two (*T. heterophylla* and *T. Mertensiana*) to the western States. They are all sources of lumber, especially the American species, and are known also as "hemlock spruce". The other group consists of very poisonous herbaceous plants of the Umbelliferae, or parsley family. One of them, poison hemlock (*Conium maculatum*), is native to Europe but has become naturalized as a large branching herb in waste places of the northeastern United States. Conium juice is supposed to have been the potion used by the Athenians in killing condemned criminals and philosophers, most famous of whom were Socrates and Phocion. A similar plant, water hemlock (*Cicuta maculata*), known also as "spotted cowbane", "musquash root" and "beaver poison", is native to moist habitats in the northeastern United States. It, too, is exceedingly poisonous.

Chemicals from Oranges. Under this title there is a lengthy and abundantly illustrated article in Industrial & Engineering Chemistry 45(5): 876–890. 1953.

Labrador Tea. In cold swamps, bogs and damp woods throughout most of Canada, and extending southward into the United States as far as Pennsylvania, this low evergreen shrub (*Ledum groenlandicum*) may be recognized by the light brown wool on its twigs and on the lower side of its narrow leathery leaves, the latter being marked also by inrolled margins. During the American Revolution the leaves served as a substitute for India tea. The more northern species, *L. palustre*, has been reported preferable for this purpose, and a western species, *L. glandulosum*, is said to be poisonous.

Principles and Practice of Mushroom Culture

About 65 million pounds of mushrooms—all of one species, Agaricus campestris—are annually cultivated in the United States on specially prepared media in the near darkness of specially erected sheds. Recent developments in the technique of this highly specialized industry are reviewed in this article.

B. B. STOLLER [1]

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Introduction

Since Lambert's review in 1938 (102) there have been considerable advances in the mushroom-growing industry. Synthetic composts are coming increasingly into commercial use; the yields therefrom are as high as from horse manure compost. Manures formerly rejected as unsuitable, such as racetrack and even cattle manure, are now being modified and supplemented so that high yields are obtained. The duration of composting and of cropping are being reduced so that two, three or more crops may be harvested annually. More efficient growers are harvesting two or three pounds per square foot of bed area or five to seven pounds per surface area annually. While some of these advances stem directly from results of research in the mushroom field, a good share is derived from the recent developments in new insecticides, fungicides and agricultural engineering. The

arrival of the tractor-loader and the adaptation of other machinery for turning composts are making it possible to increasingly mechanize and industrialize mushroom culture.

This review will be limited generally to the period after 1938, although considered, too, will be a dozen earlier references which have been omitted, or require further analysis, or have a special bearing on the discussion of later research. The aim in this review is to critically evaluate the results of research for future investigations. The style, it is hoped, differs only in degree from Lambert's lucid ability to raise "problems" (102).

Since 1938 a number of diverse publications on mushroom culture have appeared, although none approaches the requirements of a complete review. The first International Conference on mushroom culture was held at Peterborough, England, in 1950. The proceedings of this conference have been published (42) and constitute an important source of information on recent developments in this industry. Lambert's brief comments (106) at the introduction of the proceedings touch on some of the high points of research in the mushroom field during the past 20 years. A journal devoted exclusively to the mushroom industry has been established (4). While it is essentially a trade journal and the official publication of the Mushroom Growers Association of Great Britain, under the congenial influence of its editor, Mr. Fred C. Atkins, it is becoming a vehicle for publication of original investigations. Edwards' report (41) on current research was too short for an adequate statement of developments, and tended to leave a number of misapprehensions. Revised editions of publications on mushroom culture have been issued by the governments of the U.S. and Great Britain. The one (104) contains an adequate discussion of mushroom growing for the practical grower. The other (131), more recent, refers to later developments; besides providing a comprehensive survey of the subject for the practical grower, it contains excellent informative photographs, appendices pertaining to special aspects of mushroom research and an up-to-date bibliography. As a medium for advertising their spawn and presumably as a service to the grower, spawnmakers have deluged growers with revised editions of handbooks on mushroom-growing (31, 111, 146, 129, 97). Judging by the three examined (111, 146, 97), it seems that these booklets are written, supposedly, in the best business interests of the particular spawnmaker-publisher. Atkins' book (6) appears to be a popular composition; photographs depicting some ten abnormal types of sporophore growth are interesting. Recent textbooks on the physiology and biochemistry of fungi (213, 59, 71, 115) may be useful, although specific references therein to the cultivated mushroom are few and insignificant. An authoritative text on mushroom culture, in which the results of research are fully, accurately and concisely described and honestly accredited, still remains to be written.

Spore Germination

Some investigators (102, 95, 178) find that the percentage of spores germinating and forming colonies is still very small; others (87, 88, 35) claim 30% to 95% germination. Kehl (87) found only extracts of malt and spawn satisfactory for germinating spores. In order to obtain 95% germination on these media, the spores had to be treated with a "disinfectant" which "stimulated" them to germinate. Of the many disinfectants tested, chloroform was most effective; steeping spores for four hours in chloroform resulted in 95% germination. Since the spores continued to germinate over a period of a month or

longer, so that the colonies formed by the spores which germinated earlier tended to conceal the spores germinating later, an accurate count was not possible; the 95% germination after 35 days, or even 73% after 25 days, were made "very approximately". No mention is made of a count of the spores prior to plating them out to record the percentage forming colonies. In a subsequent paper Kehl (88) observed that when spores were steeped in a solution of the stigma mucus of the orchid and equal parts of water, 40% of the spores remained uncontaminated, and of these 100% germinated.

In contrast to such excellent claims of spore germination, Stoller (178) obtained a maximum of 4% colonies on malt agar plus KH_2PO_4, from a suspension of spores in which the spores were counted with a Levy haemocytometer. Although the percent of colonies was low, about 25% of the spores in the suspension of distilled water were observed to form germ tubes (germinated?) after seven days. Kehl's results, however, were confirmed, in part, by deZeeuw (35) who found that spores germinated 30% to 50% in an agar medium prepared from cooked mushroom mycelium. Observation was made in hanging drops; no reference is made to actually plating out the spores. It has been known for some time that the mycelium stimulates spore germination (102, 95, 35); it is possible that enzymes (oxidases) of the mycelium oxidize toxic substances in the medium (116). Kehl's reference to stigma mucus might indicate that other enzymes, such as proteinases or cytases, might also be effective. If Kehl's methods can be confirmed by use of a haemocytometer to count the spores, then considerable progress may be expected in spore germination.

Spawn Making

Spawn making was once a highly secretive procedure, but the method is now well known and has been published in various countries (160, 219, 194, 207). The first modern advance in commercial spawn making was by germinating spores in bottles of sterilized washed horse manure—the pure culture bottle spawn. While this process was not published because of business reasons when it came into use in 1920 in the U.S., it is generally known that Mr. L. F. Lambert of Coatesville, Pa., introduced the method; the process, however, was practiced in France since the 1890's (102). The next important advance was the "grain spawn" patented by Sinden (160). Its advantages, compared to manure spawn, are ease of preparation, many more centers of inoculation and rapid growth of the mycelium from the kernel. On the other hand, it attracts rodents which tear up the beds, and it is much more susceptible to destruction by ammonia volatilizing from beds (178). When these conditions are corrected, use of grain spawn facilitates inoculation of spawn in the tray system (162). Spawn made with substrates of chopped tobacco stems (145) or other materials of discrete particles similarly have the advantages of ease of distribution and inoculation as grain.

With regard to the manure spawn, it appears that spawnmakers prefer a heavy, dense, white growth of the mycelium rather than the comparatively translucent mycelium and the reddish appearance of the substrate. According to Steinberg (168), translucent mycelium may be associated with injury of chemicals, whereas an opaque growth is related to better nutrition. The reddish hue appears to be due to secretion of acids by the mycelium, especially oxalic acid (17, 56, 154). Addition of $CaCO_3$ to grain (160) completely transforms the translucent-like mycelium to an opaque dense white growth, but the same effect is not produced by adding $CaCO_3$ to washed manure. When salts of Al, Fe and Mn are added to the ma-

nure along with lime (172), however, the spawn is dense, white—milky—in appearance. Use of Al and Fe salts to flocculate organic matter in the purification of water is well known; the function of these salts is probably the same when mixed with manure. The gypsum recommended as a flocculent by Pizer (139) is not so effective as are Al and Fe salts (17). Microscopic examination of the " milky spawn " (172) reveals innumerable crystals incrusting the hyphae; presumably these are calcium oxalate. The oxalate has been correlated roughly with density of mycelial growth (56), shown to serve as a source of carbon (198) and as a reserve material (17). In these grain and manure substrates mentioned, the pH is adjusted on the alkaline side rather than made slightly acid as indicated by Lambert (102).

In connection with his work on the production of mushroom mycelium in submerged culture, Humfeld (81) attempted to spawn mushroom beds by spraying the liquid culture on the compost. The mycelium grew very rapidly, forming a white mat over the surface of the bed overnight, and then disappeared as quickly. In a similar test by Dr. Hoogerheide of the Pabst Laboratories on compost furnished by the writer, it was observed that the mushroom mycelium made a slight initial growth and then was overcome by swarms of bacteria. Humfeld also attempted to spawn beds with grain spawn prepared with mycelium from submerged culture. The mycelium formed an excellent fluffy growth on the grain, but again was unable to grow into the compost. He concludes that " these strains are spontaneous mutants, which can reproduce themselves only vegetatively in pure culture medium ".

Kligman (95) studied four operations pertaining to making spawn: sub-culturing, stability of spore and tissue cultures, spawn substrates and age of mycelium. In 28 sub-cultures made in rapid succession at six-day intervals on potato-dextrose agar of mycelia from mono- and multi-sporous and tissue cultures, no changes were apparent in the appearance of the mycelia, nor was production of sporophores affected. In a series of sub-cultures on rye grain, a single mutant appeared in a monosporous mycelium after subculturing 12 times. Kligman summarizes that the mycelium can be sub-cultured indefinitely, and at the same time, in reference to grain, he states that mutants probably increase with continued sub-culturing. His results apply strictly to a rapid succession of transfers and not necessarily to so many transfers made at longer intervals. Since detection of degeneration of mycelium depends upon sporophore growth and it is evident that abnormalities can occur, the practical method involves testing sub-cultures by growing sporophores—a lengthy procedure.

He concludes that mycelia from tissue of the sporophore are not less stable than mycelia derived from spores and may be used equally well for making spawn. But tissues removed from sporophores may contain incipient disease or unknown virus infection; these are less likely to be transmitted by spores. Growth of mycelia of tissue cultures is retarded, compared to cultures from spores on a malt agar to which 1% ethyl alcohol is added (185); that is, under certain conditions, tissue cultures grow slower. Enzymes from mycelium appear to be different from those derived from sporophores (64, 116A). Kligman finds that aging of the mycelium does not result in variations with limits of two years. In contrast, the writer (185) observed that grain spawn stored eight months at 35° F. usually failed to grow on malt agar containing phenol, aniline, hydroquinone, etc. The response of age of mycelium to the amounts of ethyl alcohol added to malt agar was so uni-

form that this procedure could be employed in estimating the age. These reactions indicate that as the mycelium ages, there is a decline in its oxidase activity or ability to resist products of this activity. While it is well known that good yields have been obtained with spawn two to five years old, accurate data on productivity and susceptibility to disease are not available.

Kligman (95) tested ten different substrates for preparing spawn; of these only spawn on cotton seed hulls grew slower and resulted in poor yields. He concluded that strains are not fundamentally altered by the substrates on which the mycelium is grown. The "liquid spawn" previously mentioned is certainly an instance where strains are entirely altered by the nature of the substrate. It is not possible to enter into a discussion of "enzyme adaptations", well known to bacteriologists (170). Suffice it to state that enzyme adaptation is due to "natural selection working on a spontaneous variation in the culture" or due to the nature of the substrate and environment (170). In Kligman's limited studies he observed a different spawn effect on cotton hulls, sectoring on rye grain, a mutant after 12 sub-cultures on rye grain; it is possible that indefinite subculturing on grain would induce further mutations. In his discussion on techniques in spawn making, Kligman has failed to consider the adaptations and inheritances of enzymatic and biochemical characteristics.

Cytological and Genetical Characteristics

Fructification with Single Cells. In transferring cultures after storage for three to six months, Kligman (94) noticed that some hyphal elements became detached. The separated or detached cells were found to be very variable in size and shape, tending to be linear instead of spherical. These detached cells were always fertile and, when prepared as spawn, resulted in a normal crop of sporophores. Humfeld (81) also observed an abundance of these detached cells described by Kligman, in submerged culture medium containing little Ca and adjusted at pH 4.5 (83). In order to find a possible explanation for the separation of these cells it is important to note that they arise in old cultures or in a relatively acid media containing little Ca, that is, under conditions in which the oxalic acid formed by the mycelium, previously mentioned, might accumulate freely. The significance of this acid accumulating freely is that oxalic acid, added by Northcraft (135) to media in which plant tissue grew, induced a separation of the cells of the tissue; in this way single viable cells were obtained. The oxalic acid precipitated the calcium—"the cementing material"—so that the cells separated. The cells were of various sizes and shapes, but mostly "bean or banana-shaped"—like spores. If Northcraft's experiment on detaching cells of plant tissue is applicable to the separation of mycelial cells, there is no need to refer to them as "chlamydospores" or "secondary spores" (94, 155); they are merely detached cells and should be so designated.

Two- and Four-spored Varieties. There is agreement in the recent literature (99, 95, 153) that the cultivated two-spored *A. campestris* is morphologically and physiologically distinct from the wild four-spored variety. One is not a "form" of the other; Kligman (95) prefers to designate it as "variety", whereas Sarazin (153) refers to it as "distinct species". Considering that the nuclear phenomena of both correspond (99, 95), the designation "variety" should be sufficient.

The physiological difference is attributed by all (99, 95, 153) to the inability of the four-spored variety to grow on

composted manure unless the manure is diluted with about 50% soil (99) or diluted with straw and 33% soil (153). Besides, the two-spored variety is rarely found in meadows (95), the common habitat of the other variety, although Sarazin (153) set spawn in meadows and obtained sporophores of the two-spored variety on the site of inoculation. It appears, then, that the two-spored variety can grow in meadows but has become adapted to grow on a more concentrated medium. Adding soil to compost could be regarded as a means for reducing the oxidase activity, or its secondary products, of the four-spored variety, just as may occur in the casing soil with the cultivated variety. The two-spored variety may have been derived from the four-spored variety by an enzymatic adaptation, previously discussed; the answer could be determined experimentally by the usual technique in working with enzymatic and biochemical adaptations. That adaptations of *A. campestris* can occur is clearly evident from Humfeld's experiments (81). In fact, by selecting spores on the basis of enzyme adaptations or substrates, the four-spored variety may offer possibilities for producing new strains, even though it is regarded as homothallic by Klushnikova (99).

Sexuality. In order to breed new varieties of mushrooms, to acquire certain advantages, it is necessary to understand their sexuality. Buller (16) suggests that if *A. campestris* is found to be heterothallic, breeding new varieties by crossing is possible; but that if it were homothallic, such a program could not be undertaken. " Homothallic species may be looked upon as sexually degenerate " (16). Buller proposes that to determine whether *A. campestris* is homo- or heterothallic it is necessary to find out, using Hirmer's method, whether or not conjugate nuclear division takes place in the hyphae of monosporous mycelia. If such division occurs, then *A. campestris* is homothallic; if not, it is heterothallic—provided that " confirmation of the conclusion in respect to heterothallism could be obtained by mating monosporous mycelia in all possible ways, etc." (16).

Kligman (95) has made an excellent investigation of Buller's proposition, although he mistakes it for a generalization, thus entailing unnecessary arguments. In a recapitulation of the cytology of the cultivated mushroom, his results were found to agree closely with those of previous investigators, but Hirmer's observations could not be confirmed on conjugate nuclear division; *i.e.*, the nuclei did not divide simultaneously nor side by side with their long axis parallel; instead, they divided one above the other. Since conjugate division was absent, Buller would consider it heterothallic—if it could be confirmed, as stated above, by mating monosporous mycelia. Kligman (95) has shown that production of new varieties by crossing is severely limited. Furthermore, he has shown that even when crossing " sterile mycelia " which might be regarded as equivalent to the unisexual condition, there were few successful " crosses ", and from these, few mushrooms were produced. There was no evidence for a mutual exchange and migration of nuclei as occurs when unisexual strains of heterothallic species are mated. So it seems reasonable for Kligman to conclude, despite the atypical behavior of the nuclei, that *A. campestris* may be designated as homothallic.

Kligman (95) showed, too, that *A. campestris* not only is in the sexually degenerate state of homothallic species but may be regarded as non-sexual. Circumstantial evidence indicates that the nuclei fusing in the basidium are not of opposite sex. Direct proof will depend upon picking off and germinating spores from three-spored basidia; at

present this procedure is not possible. For the present the fusion of nuclei in the basidium represents a vestigial trait that has lost its genetical significance (95). The derivation of essential substances or metabolites ordinarily procured by means of synthetic biochemical reactions arising as a result of the interactions occurring during the sexual process, seems to have been displaced by a physiological dependency on substrates sufficient in these essential metabolites, such as may be served very well by manure, *i.e.*, a complex mixture of bacterial, fungal, plant and animal products.

As physiological factors enter into the qualifications of a morphological distinction, such as homo- and heterothallism, the number of particulars increases until order is reestablished by engendering a new principle or generalization. In a recent review of heterothallism in fungi, Whitehouse (209) shows that there is a morphological and a physiological heterothallism; that the latter consists of several sub-groups. Then there is a primary and secondary homothallism, besides an intermediate type—a partial heterothallism. It may be expected that there is a physiological basis behind the " sterile mycelia ". A physiological basis is necessary to understand sex of *A. campestris*.

Sterile Mycelia and Hybridization. Kligman has made an extensive study of the relation of monosporous mycelia to fructification. Like Sinden (160-A) he found that 25% of the monosporous mycelia were sterile, that is, failed to produce sporophores. In the attempt to mate sterile mycelia, only seven out of 55 crosses were successful, but very few mushrooms were produced in each case. Sporophores were produced only from mycelia removed at a distinct line of demarcation or junction of the two sterile colonies; that is, there was no mutual exchange or migration of nuclei as occurs in heterothallic species. The brown and white forms of *A. campestris* remained distinct despite diverse attempts to " hybridize " them. In the attempt to pair sterile mycelia of these two forms, it was observed that the same brown mycelia crossed with a white form to give a brown mushroom, and again with another white form to give a white mushroom. These two white forms had been crossed with each other, so he concluded that sexuality alone is probably not responsible for the successful crosses. Hybrids could not be detected among the sporophores arising from crossing, so that this method does not offer a means of producing new variations.

Cultural Characteristics of Colonies. From among 16 monosporous mycelia, Kligman (95) classified five colonies with different cultural characteristics. Stoller (178), working with 105 mycelia (43 of these were irradiated), classified 18 colonies according to cultural characteristics, although seven were subordinate to the others. Besides the appressed and fluffy colonies mentioned by Kligman, other colony characteristics observed were concentric ring formation, translucent mycelium, reddish-purple mycelium, and slow growing completely appressed colonies. Kligman observed that sectoring, a tendency to form rapidly growing luxuriant white mycelium, was frequent in rye grain but absent in manure cultures. He seems to imply that sectors could be induced in any monosporous mycelia on potato-dextrose agar. The fact that he found that sectoring did not occur in malt extract agar may be the explanation for Stoller's finding that sectoring is restricted to certain types by using malt agar exclusively as a medium. None of these cultural characteristics mentioned by either investigator is apparent on a manure substrate, so they are probably related to nutritional factors. The fantail appearance and rapid growth of sectors resemble, in a way, the mycelium

as it enters and grows through the casing soil, so it might be entertained that sectors are an abortive attempt at fructification.

Kligman (95) conducted productivity tests with monosporous mycelia; he observed that cultural characteristics, such as growth rate and appearance, could not be correlated with productivity. The only criterion for separating fertile monosporous mycelia is production of sporophores. Sarazin (151), on the other hand, claims that the most vigorously growing mycelia isolated produced mushrooms a week earlier and formed mushrooms with large caps and stout stems; after repeated transfers (*sic*) the strain reverted to the long stem kind. Without physiological criteria, the usefulness of isolating monosporous mycelia for new strains is doubtful. As in yeast genetics, it is necessary to change over to a study of inheritance of enzymatic and biochemical characteristics (144).

Irradiation of Spores. In an attempt to induce mutations, mushroom spores were subjected to ultra-violet radiation (178). Although 4000 spores were plated out per Petri dish, only one or two colonies formed after irradiation for 20 to 35 minutes, whereas the colonies were relatively numerous after irradiation for 15 minutes; accordingly it was assumed that colonies arising after irradiation for 20 minutes were affected. Colonies from irradiated spores grew very slowly in some types; others grew rapidly and were much more inclined than non-radiated spores to form sectors and other irregularities. There was less tendency for fluffy mycelium and concentric ring formation among the mycelia of irradiated spores. The effect of irradiation of spores on productivity was not tested. Others (166) attempted to induce variations by adding uranium nitrate to potato-dextrose agar. It is claimed that the mutants induced produced five to seven times as much mycelium as original stock, and affected earlier production. To date, the writer has not heard of confirmation of these claims.

Physiology of Mycelial Growth

Environmental Factors. TEMPERATURE. Lambert (104) presents a photograph in which it appears there is linear increase in the rapidity of mycelial growth from 50° F. to a maximum at 77° F. Treschow (198) repeated Lambert's experiments in test tubes; at the same time experiments were conducted on the weight of mycelium grown on the surface of a fluid medium. Results both on compost and fluid media indicate that there is a sharp maximum at 75° F. He states "the mycelium is very sensitive to the temperature, and its optimal growth is really limited to a very narrow range of temperature". The mycelium in the fluid cultures had access to air, but not in the test tubes, which were corked and sealed with paraffin. These results on a sharp optimum are immensely significant for recent developments in the short and mechanized systems of mushroom culture.

AERATION. In his experiments on the effect of temperature, Treschow states that growth did not appear to be hampered by lack of a continual renewal of air, but the effect of aeration in this otherwise meticulous work was not actually tested. Sinden's claim is even more emphatic: "air is unnecessary during the mycelial growing stage" (162). It would seem, though, that oxygen is consumed and CO_2 exhaled in order to account for a 10% loss in total dry weight during growth of the mycelium on sterilized manure for 50 days (205-A). In submerged culture the rate of growth of the mycelium was increased when the rate of aeration was augmented (83). Mader (119) observed that mycelial growth was excellent in trays placed in an enclosed room, not sealed air-tight.

But the yield was reduced one-half compared to that of mycelium in trays placed in a ventilated room. If both mycelial growth and fructification were attempted in the closed room, no mushrooms at all appeared. It is evident that the mycelium can grow where air exchange is reduced to a mere diffusion. Although it is theoretically possible for the mycelium to utilize the CO_2 of its respiration for synthesis, there is no proof that it can grow without access to oxygen. But the practical question under consideration is Sinden's claim (162) that a normal crop can be produced when the mycelium is grown in a confined space, whereas Mader's experimental proof is that under these conditions the yield is reduced by one-half. This contradiction is of practical importance and should be resolved.

pH AND MOISTURE. In fluid media the largest production of mycelia occurs at the initial pH 6.8 (198). The rate of growth on manure compost adjusted with H_2SO_4 was fastest at pH 6.9, little change $\pm$ 0.4 pH (198). By saturating manure compost with Ca ions, then drying and adjusting pH with either HCl or NaOH, dense growth obtained at pH 6.2 to 8.5, but rate of growth was maximum between pH 6.0 and 6.6 (140). By washing manure with Ca ions it became poorly buffered so that the pH was reduced to 4.0 to 5.0 by mycelial growth (140). The buffering capacity is inextricably associated with optimum pH (198). When the concentration of the buffer is optimum for growth, it is too low to maintain constant pH; hence it is impractical to determine optimum pH (198).

The pH values may be altered by as much as one unit by increasing the moisture from 50% to 66% (100% to 200% dry wt.) (140). As might be expected, pH increases directly with the moisture content, probably because more of the alkaline ions, K and Na, are brought into solution. Addition of gypsum tends to lower the pH (140), possibly because, as the Ca is exchanged and adsorbed, the neutral salts forming K_2SO_4, Na_2SO_4 and $MgSO_4$ are not so alkaline as their carbonates. The ammonium ion is reflected chiefly in the effect of the volatile ammonia on mycelial growth. The pH of a solution of NH_4HCO_3 is the same whether the normality is 0.1 or 0.003, but the NH_3 volatilizing at 0.1 *N* is much more rapid than at lower concentration (178). For this reason ammoniation would not be detected by a pH test; but it can be detected by suspending strips of filter paper in which phenol red is absorbed, above compost in bottles (178). It appears, then, that the buffering capacity, flocculating agents, moisture content and ammoniation may be more significant in determining growth than the apparent pH. The optimum moisture content, while suggested as 61% (102), is a resultant of the interaction of pH, temperature, aeration, dispersion of colloids, degree of composting and nature of fibrous materials.

Nutritive Factors. MINERAL COMPOSITION OF MYCELIUM. It has not been possible to separate the mycelium from the substrate of *A. campestris*. The mycelium of a wood-destroying fleshy fungus appears to be more easily differentiated (68). The analysis of substrate, mycelium and sporophore of *Merulins lacrimons* is shown in Table I. Although this analysis was made in 1885, the close similarity of the data regarding the sporophore with recent analyses (60, 2) would indicate that it is reliable. The mineral composition of sporophores of diverse fungi, including *A. campestris*, has been shown to be generally similar (60), so the same may be assumed for the relative composition of their mycelia. The increase in Al, Fe and Mn in the mycelium is very interesting. The large amount of Ca in the mycelium may be

present as calcium oxalate. The composition of the sporophore is typified by a large increase in K and P, and a decrease of all the other elements.

MINERAL REQUIREMENTS. Ca has been depicted as an essential nutrient for the mycelium (198), as an antagonist to K and Mg (198), as a flocculent when applied as a relatively soluble salt (139, 143), and as a precipitant of oxalic acid (172). The essentiality of Ca in nutrition of fungi has been verified (169). Treschow (198) suggests that the influence of Ca as an antagonist is the basis for the flocculent effect of Ca on the grounds that flocculation with other ions has not the same effect. It has been shown, however, that other ions—Fe, Al, Mn—can flocculate the organic colloids of the compost (172, 17). It is known in plant physiology that some degree of antagonism exists between almost any pair of salts. For example, addition of K_2CO_3 to sterilized manure compost prevents mycelial growth, whereas when $Al_2(SO_4)_3$ is added at the same time, good growth occurs (172). Whether Ca is designated as flocculent or antagonist, it has the same function—namely, to exchange or "antagonize" the ions Na, K, NH_4 and Mg which produce a dispersion of colloids of compost.

In experiments with different Ca compounds for flocculent action, Pizer and Thompson (143) found $Ca(OH)_2$ and $CaCO_3$ ineffective, $CaCl_2$ the fastest acting flocculent, $CaSO_4$ or gypsum an effective flocculent and also valuable for regulating the capacity of the compost to absorb and retain water, and superphosphate (a mixture of $CaSO_4$ and $CaHPO_4$) suitable also, since it is moderately soluble in water. The fact that the cut stems of mushrooms turn brown when grown on compost with $CaCl_2$ is indirect evidence of the absorption of Cl ions.

Although use of gypsum in mushroom composts and the explanation of the action and effectiveness of gypsum are distinctly Pizer's contributions, there is, nevertheless, earlier reference to the same use. Some 30 years before Pizer's publication, Schuler (156) observed: "Zur Champignonzucht is der Gips (schwefelsaurer Kalk) zur empfehlen". He recommended mixing 90 to 100 pounds of gypsum per ton of manure. The gypsum was to be utilized for reacting with the ammonia evolving during composting, by a double decomposition reaction ($CaSO_4 + (NH_4)_2CO_3 = (NH_4)_2SO_4 + CaCO_3$). While this reaction may occur to some extent, more likely the acidity of the free SO_4 ion, after the Ca ion is adsorbed, tends to suppress the "urea" bacteria, thereby reducing ammoniation. This phase will

TABLE I

MINERAL COMPOSITION OF MYCELIUM AND SPOROPHORES

	Healthy * wood	Mycelium * (wood destroyed) *M. lacrimons*	Sporophores	
			M. Lacrimons *	*A. campestris* †
K_2O	8.6	7.2	56.1	44.0
Na_2O	0.9	5.5	1.6	2.3
MgO	4.8	1.8	Trace	0.2
CaO	36.6	31.0	0.4	1.3
$Fe_2O_3 + Al_2O_3$	4.8	11.2	4.0	2.6
Mn_3O_4	0.8	4.4	Trace	Trace
SO_3	2.9	4.0	2.6	3.0
P_2O_5	2.6	0.9	25.0	24.3
Total ash	0.2	1.5	9.7	11.5

* Modified after Hasenohrl and Zellner (68). † Friese (60).

be discussed later. Flocculation of humus goes back to the work of Oden in 1919 (150); Ca salts were found particularly effective in flocculating humus dispersed by Na, K and NH_4 ions. Pizer's contribution is the insight to solve a problem; a knowledge of pertinent priorities helps to reaffirm conclusions.

TRACE ELEMENTS. It is evident from Table I that trace elements are constituents of the mycelium. The mineral requirements for maximum mycelial growth, even on a dry weight basis, are not easily shown because the requirements are so minute. Treschow (198) doubts that any significance can be attached to Bechman's results with trace elements because concentrations of nutrient solutions which he used were too high. Treschow did not observe any effect of Cu, Zn and Mn on growth of mycelium, although a trace of Fe was required. Stoller (173) proposed the use of such trivalent ions as Al, Fe and Mn to improve the growth of the mycelium, not only because they were effective flocculents but also because they were present in large amounts in mycelium, as shown in Table I.

CARBOHYDRATES. In pure culture on fluid medium, Treschow (198) observed that mycelial growth is particularly vigorous with xylan; the dry weight of mycelium was greater with xylan than with other sources of carbon. Also mentioned as C sources were calcium oxalate, calcium malate and calcium pectinate. The utilization of calcium oxalate is especially interesting because crystals of calcium oxalate have been observed incrusting the hyphae. His comments on composting which pertains to the utilization of xylan will be discussed later.

NITROGEN SOURCES. Treschow (198) found that nitrate " could not be utilized as a source of nitrogen " by the mycelium. This statement is surprising, since nitrates have served as N sources in synthetic composts (177, 40). Only one salt, KNO_3, was tested; it would appear that $Ca(NO_3)_2$ should have been tried, since he found K toxic. Lack of molybdenum (188) or other elements (202, 121) required for the reduction of nitrate may have influenced the negative results. Inorganic sources of N, such as $(NH_4)_2SO_4$ and NH_4NO_3, were not so suitable as organic sources on the basis of weight of mycelium produced. Asparagine was the best source of N. Good growth occurred even with 1.05% N as asparagine, but a strong toxic effect was exerted by concentrations exceeding 0.063% N as NH_4 ions. His conclusion that this toxic effect must be specific of the NH_4 ion is hardly warranted by the fact that with other NH_4 salts, such as ferric ammonium citrate, growth was as good as with asparagine. Good mycelial growth and a yield of 1.84 pounds per square foot was obtained with a snythetic compost prepared with 0.5% N as $(NH_4)_2SO_4$ (47.5 lbs. $(NH_4)_2SO_4$ per ton—wet weight) (177). In order to use $(NH_4)_2SO_4$ as a N source, cheese whey (70% lactose) had to be added at the same time.

Metabolic Processes. The mycelium is assumed to utilize solid, structural, water-insoluble materials like lignin, cellulose, hemicellulose and proteins, or insoluble trace elements because these substances promote growth in pure culture or because, by analysis, a reduction of these is observed after growth. How are these substances decomposed and assimilated by the mycelium? Enzymes are known to accelerate and even initiate metabolic processes, but the question is, Which processes are accelerated and how are the end products absorbed by the mycelium? Absorption by the mycelium might be explained by the usual " soil solution theory ", but the assumption would have to be made that only water-soluble substances are absorbed; it has been demonstrated that this is improbable, at least for water-insoluble trace

elements and inexchangeable elements (85). On the other hand, according to the " contact exchange theory " (85), the plant roots feed directly upon the solid phase; the reactions take place without the participation of free electrolytes. The theory embraces all colloidal systems, all ionic surfaces, not only soil colloids and plant roots. According to this theory the adsorbed cations on colloidal clay or other colloidal complexes are directly (by-passing the soil solution) replaced by or exchanged for hydrogen ions furnished by organic acids synthesized by the roots (or mycelium). This theory has been substantiated recently by a study on absorption of radioactive zirconium and niobium by plant roots (203). These ions, Zr and Nb, are readily absorbed by clay and soil, but resist leaching, even with 0.1 *N* H_2SO_4 or 0.1 *N* HCl; however, they are readily removed with oxalic acid of the same normality. Oxalic acid is more efficient than other organic acids at the same concentration in releasing absorbed cations.

It is suggested that the H ions of the oxalic and possibly other acids, secreted by the mycelium, release and are exchanged for the cations absorbed on the colloidal complexes by direct contact of the mycelium with the solid surface. Now, returning to the question, What process is accelerated by the enzymes? The H ions formed by the oxalic acid, facilitated by enzyme action, tend to disrupt or decompose the water-insoluble, solid, structural substances of the compost so that the smaller molecules formed are absorbed by direct contact. The mechanism, then, constitutes an enzyme-mediated hydrogenation. A pertinent analogy is afforded by the recently developed process for hydrogenation of the by-product lignin in the paper industry. The lignin is hydrogenated by saturating it with H at enormous pressure at 325° C. with a copper chromite catalyst (67-A). Enzymes, as distinguished from chemical catalysts, bring out such reactions at ordinary temperature and pressure. Among the many products arising from the hydrogenation of lignin, one is catechol which, as will be shown later, is related to mechanisms for fructification and disease resistance. The significance of the contact exchange theory in unraveling the metabolic processes of the mycelium has been indicated, but conjectures are notorious for their habit of being disproved by actual experimentation. Yet some kind of comparison, conjecture, formulation or hypothesis—call it what you will—must be envisioned as a basis for initiating experiments.

Enzymatic Activity of Mycelium. Quite a few investigations have been performed on isolating enzymes from sporophores (89, 192, 193, 132, 65, 204), but only two attempts have been made to isolate an enzyme from mycelia (63, 64, 116-A). The enzyme was extracted from bottle manure spawn (64). It was stable only in acid solution (pH 4.0–6.5) and was considered not identical with the polyphenol oxidases isolated from sporophores because it was not activated by Cu ions. It was found to oxidize lignin and various phenolic substances. Its activity was enhanced by $MnSO_4$ and slowed down by $MgSO_4$, according to Dion (36) who found the same effect produced by an extracellular polyphenol oxidase which was isolated from a submerged culture of *Polyporus versicolor*.

Dion (36) suggests that the oxidase of *P. versicolor* may be identical with the enzyme from mushroom spawn. It appears to oxidize lignins to ortho-quinones. The color changes produced by the enzyme preparation acting on some 20 phenolic substances are interpreted as formations of ortho- or para-quinone. This investigation might be considered a confirmation of experiments conducted by the writer over ten years ago (174,

175, 177), in which 71 phenolic substances were tested by the color changes formed in malt agar as a result of mushroom mycelial growth. Besides the many color changes, crystals or " needles " were formed with two chemicals; these crystals provide, it seems, more conclusive evidence that quinones were engendered. According to Karrer (86), both hydrocoerulignon and 1,3-pyrogallol dimethyl ether are oxidized to coerulignon (a quinone) which has crystals with a steel-blue color. In agar medium crystals were formed with a purple to black color, darkened possibly by impurities in the agar. The oxidation of the ether occurred in two steps, the crystals being preceded by an orange ' halo '. As far as is known, this is the only instance in which these chemicals were employed to demonstrate oxidations by living cells or enzymes The color reactions with barium diphenylamine sulfonate and erioglaucin, which have high oxidation potentials, would tend to indicate the strong oxidizing capacity of the mycelium.

Methods of Growing Mycelium for Investigation. Previous methods of growing mycelium pertained to observing rate of growth on an agar or silica gel medium or measuring weight of mycelium grown on a fluid surface or submerged. A medium of fibrous particles would approximate the physical condition of the compost. Burrows (17-A) proposed an aerobic medium based on milled transparent cellulose shavings dyed black for observation of mycelial growth. After the cellulose is dyed, washed and dried, it is ground to pass any mesh, preferably one millimeter, and is then filled into test tubes. In preliminary experiments excellent agreement in growth rates is claimed. A technique developed by the writer (185) may be described as a particle-shake-bottle method. Thoroughly cleaned and ground (to size of corn grains) corn cobs at the rate of four ounces of cobs, eight ounces of water and various nutrients were filled into the usual spawn bottle and sterilized. Every time the spawn grew throughout the corn cob medium, the bottles were shaken in order to break up the mycelial growth and observe how many times the mycelium would grow back. Intervals of about a week were allowed between the times the bottles were shaken. Only preliminary experiments were performed; however, it was observed that when the N sources were soybean meal, calcium caesinate or mono-ammonium caesinate, the mycelium failed to grow back after the bottles were shaken twice or thrice; the particles appeared as if they had never been inoculated. It was thought that some lysis or bacteriophage was encountered, but on transferring the particles to agar or grain, the mycelium grew normally. It is possible that a detachment of cells occurred as described by Kligman (94) and Humfeld (81) mentioned previously; however, no microscopic examinations were made. In a reference to " mycophagy " it is stated that lysis of fungi depends on the nature of the substrate (201). This method may be useful in the study of lysis, nutrient requirements and the truffle disease, as will be mentioned later.

Composition of Composts and Cropping Experiments

Formulation of Composts. The first attempt at formulating " artificial " composts for mushroom culture, employing a fermentation process, was based essentially on the method of Richard and Hutchinson (147, 102). It consisted in providing a suitable source of N and moisture to induce microbial decomposition of straw under aerobic conditions. The yield of mushrooms from such composts was much less than that from the usual composts of horse manure. For this reason, Stoller (171, 186) proposed

that horse manure be analyzed for its major constituents in order to find other ingredients that might influence the yield. Later (173, 177) it occurred that an analysis of the ash of the sporophore might also give some indication of the elements required. From each of these analyses it was evident that nitrogen (N), phosphoric acid (P_2O_5) and potash (K_2O), or briefly NPK, were the chief constituents. Accordingly a hypothesis was established that a compost should contain NPK in the proportion present in manure (2:1:2.7) or in the sporophore (2.7:1:1.8). As a result of extensive experimentation, using spent licorice roots, it was found that the proportions of NPK in the sporophore was a closer approach to the requirements; that is, somewhat more N and less K_2O than are present in horse manure. Therefore it was proposed that a ton of "synthetic" compost with 70% moisture should contain about 13 pounds N, four pounds P_2O_5 and ten pounds K_2O. This method was evaluated as a means for selecting the least expensive ingredients compared to yield. Yields of 2.5 to 2.9 pounds per square foot were obtained with licorice root composts on a commercial scale—(several houses of 5,000 sq. ft.) in 1938 (177).

A method of preparing composts based on an analysis of horse manure was patented in Germany in 1891 by Herfurth (76). Even at that early time an "Ersatz" was proposed for composted manure, consisting of a solution of $NaNO_3$, $(NH_4)_2SO_4$ and K_2HPO_4 in "rain water", to be added to chopped straw and then mixed with peat; the amount of peat was equal to six times the quantity of straw. No recourse was made to fermentation, which would have been difficult anyway, with so much peat. The nutrient solution was added every three days throughout cropping.

Actually Stoller's method amounts to an application to mushroom culture of fertilizer practise well known in agriculture. The controversy of the significance of soil analysis and ash analysis of plants for determining the mineral and N requirements has waxed and waned since the arguments of Liebig, Laws and Gilbert in the eighteen-forties. The current agricultural practise of estimating the nutrient requirements in terms of NPK are equally applicable to mushroom culture. Among other investigators of substituted or "synthetic" composts, one (44) is handling his data in terms of such fertilizer practise, while others (104, 161, 21) still make specific mixtures independent of analyses of constituents.

Edwards (44) attempted to correlate the synthetic composts prepared by various investigators on the basis of supplements per ton of straw, without considering the NPK constituents of straw or supplements besides minerals. While such correlations avoid errors in analysis, they tend to show greater variation in the NPK constituents than is warranted by the yields. In Table II an analysis of formulas is presented, using 600 pounds of air-day sources of carbon as a common denominator—that is, the amount equivalent to a ton of fresh horse manure. Sources in which the predominant constituent by far was carbon, such as straw, grains and meals, are considered wholly as a carbon source.

The usefulness of NPK formulation may be shown in several respects, besides providing a basis for comparisons. In referring to the special nutrient requirements of sporophores, Lambert (102) observed that synthetic composts as he prepared (Table II), when mixed half and half with composted manure, produced normal yields. It is quite evident that by this mixing, the potash of his compost was almost doubled and probably accounts for the increase; subsequent experiments with K_2O substantiate this explanation (177). However, increasing the K_2O above, without refer-

ence to the N and P_2O_5 requirements, would increase the yield rleatively little. This statement brings us to the question of the " law of diminishing increments " and the " principle of limiting factors ", upon which NPK analysis is entirely dependent. Willcox (210), referring to the immense data accumulated about fertilizers and plant growth by Mitscherlich, presents a very interesting story on the applications of these rules to plant growth. For example, the yield or " fertility index " is dependent not merely upon increasing the amount of the factor present in minimum amount but also upon the multiple of all the factors: $F(N) \times F(P) \times F(K) = \text{Fertility index}$. The principle of limiting factors which influence the yield of crops, in its present-day form, was proposed by Mitscherlich in 1909 and has been stated as follows: " the increase in any crop produced by a unit increment of a deficient factor is proportional to the decrement of that factor from the maximum " (126). Until the maximum yields for mushrooms are obtained under standard conditions, as was done by Mitscherlich for many green plants, reference to " optimum " quantities of NPK has little significance.

While Sinden still lists the ingredients for his synthetic compost without NPK analysis, it is evident that in his " further studies " (161), all the NPK ingredients have been increased. In attempting to apply his formula on a commercial basis, some further changes were made (Table II). Along with his co-operator, Garzia, it was found that

TABLE II

COMPOSITION OF COMPOSTS BASED ON APPROX. 600 LBS. AIR-DRY CARBON SOURCE (EQUIVALENT TO ONE TON FRESH HORSE MANURE). AMOUNTS IN LBS.

Investigator	N	P_2O_5*	K_2O*	Sources of supplements		
				N	P_2O_5	K_2O
(Horse manure)	12.0	6.0	16.2	.....	.....	.....
Adco M (Richards and Hutchinson, 1920)	6.3	2.2	6.0	Inorg.	Super-P.	None
Demolon et al., 1937 (34-A)	6.6	1.0	6.0	Urea	None	None
Lambert, 1941 (104)	8.6	6.0	6.0	Dr. blood & urea	Super-P.	None
Stoller, 1942-3 (177)	13.0	4.0	10.0	Any source (malt sprouts, br. grains, etc.)	Any source (super-P. grain prod.)	Any source ($KC^{\cdot}$, K_2SO_4)
Stoller (Licorice roots)	13.2	3.1	9.5			
Sinden, 1946 (161)	8.3	2.8	8.5	Several (Ca cyan. & rye grain)	Grain & corn fodder	KCl
Sinden, commercial modif.	9.1	2.5	10.7	(Ca cyan. & brewers gr.)	Grain & corn fodder	KCl
Edwards, 1949 (40)	12.8	3.2	11.1	Several (dried blood)	Super-P.	KCl
Chapuis & Courtieu, 1950 (21)	6.5	5.0	11.7	Several (urea & rye flour)	KH_2PO_4	KCl

* Preferable to state P and K as P_2O_5 and K_2O, to align with fertilizer practise and commercial sale of these ingredients.

working with long straw was laborious both in turning and in filling the trays. As might be expected, the large dry kernels of wheat contributed little of their nutrients until they were ground or substituted by a crushed grain. Sinden states that in making this substitution, a large amount of N was used unintentionally because Garzia did not allow for the larger percentage of N in the brewers grains; however, an analysis by the writer shows that the increase in N was contributed, instead, by supplying 33% more Ca cyanamide. No mention was made of the fact that the K_2O was increased 23%. The best yield, with the modified formula, was 1.74 pounds per square foot, although the cropping period had to be limited to 42 days.

Edwards (39, 40, 43, 46) conducted a series of experiments in 1946–1951 on synthetic composts with long straw and several N sources, arriving at a formula (Table II) similar to Stoller's (177). The yields from his experimental synthetic composts are generally low, usually not more than one pound per square foot. In a private communication (48) he states that yields of three pounds per square foot have been picked from single beds, but that the environmental conditions of his experimental house are relatively unfavorable. Atkins (8), his cooperator on a commercial basis, has obtained excellent yields, 2.8 pounds per square foot in 11 weeks of cropping, by following his formulations. Edwards' (48) explanation for this difference in yield is that under commercial conditions there is a more favorable environment, the straw is chopped and more of it is packed into the beds. It is difficult to understand how proper mixing and testing can be done with composts of long straw, and how Edwards conducted a number of his experiments without "peak heating". Even pasteurizing for 24 hours at 120° F., especially when the beds are not packed deep, and despite the employment of insecticides, is insufficient to eradicate insects. Under these conditions, limiting factors other than the one being tested may be present. His own estimate of his results has been published recently (47).

In Table II is shown the "premiere" formula employed by Chapuis and Courtieu (21) on a small experimental basis and composted in their "mechanical mixing apparatus". They make no use of a NPK analysis, and their low content of N is not explicable. The use of rye flour will be discussed later. On a "practical" basis they recommended 0.7 to 0.9% N per ton of straw. They consider superphosphate suitable for its flocculating property, and K of little importance, although a considerable quantity was employed in their experimental tests. Thus, like their predecessors in France, Demolon et al. (34A), they consider only N important in the preparation of synthetic composts.

Technique of Expressing Yields. In cropping experiments it may be possible to eliminate many sources of variation, after establishing standards of comparison, by applying Willcox's "Inverse Yield—Nitrogen Law" (211, 212). According to this "law" the yielding ability or producing capacity possessed by a variety of plant is inversely proportional to the percentage of N contained in its total dry weight. This means that the yield will be greater when sporophores contain a smaller percentage of N. Under special conditions evidence favorable to this rule is shown by the fact that the yield was 178% greater when mushrooms contained 6.96% N than for mushrooms with 7.42% N (composts almost devoid of potash) (183). A difference of 0.46% N is immense; in Willcox's data N differences of only 0.01 to 0.02% are significant. If this method is found applicable to mushroom culture, the determination of optimum concentration of nutrients would

be facilitated. This rule will be considered further in discussing composting and C:N ratios.

Besides the environmental factors and composition of the compost, the yield varies according to the amount of compost per area. In experiments with chopped, spent licorice roots, Stoller (177) observed that the yield was almost directly proportional to the increase in quantity of compost when the depth of the beds was three, four, five and six inches; however, when the depth was increased to seven inches, there was a noticeable reduction in yield. Edwards (39), using long straw manure, obtained a large yield per quantity of material with a bed five inches deep than for a bed nine inches deep. Lambert and Ayers (108-A) indicate that the yield per quantity is more from thinner beds, but the "comparative" efficiency depends on the length of the cropping period.

TABLE III

YIELDS FROM COMPOSTS OF HORSE MANURE AND GYPSUM

Investigator	Days composting	Lbs. per sq. ft.	Sq. ft. per ton fresh manure	Lbs. per ton fresh manure
Sinden & Hauser (163)	15	1.80	236	425
Sinden & Hauser (163)	10	1.88	400	743
Lambert & Ayers (107)	14	1.7	150	225
Lambert & Ayers (108)	14?	3.5	150	500
Edwards (44)*	21	2.7	143	385

* Compost contains other ingredients besides gypsum, but not effective.

It was suggested at the International Conference in 1950 (42, 43) that wherever possible, investigators should express yields both as pounds per square foot and as pounds per ton of compost. The immense differences encountered in these forms of expressing yields is shown in Table III. An attempt to explain these differences will be made under method of composting.

Fibrous Materials. Synthetic composts are prepared with the same fibrous materials found preferable as stable bedding in manure composts, namely, rye and wheat straws. Oat or barley straw and corn stover were objectionable as bedding on account of the tendency to lose their fibrous structure when fermentation had proceeded to the stage where moisture was retained well. A study was made on subdividing various roughages by different mills, grinders and cutters (171). The aim of subdivision was to get higher temperatures in the compost pile, to retain heat better and to permit easier mixing of materials. Various methods of sub-dividing fibrous materials are practised (104, 177, 8).

Good results have been obtained by the use of other fibrous materials, *e.g.*, spent licorice roots, spent myrobalan nuts, spent oak bark (177), spent hemlock bark (178) and bark from pine, spruce and poplar removed in preparing pulp (185). Little or no fermentation is required with these materials besides a period for pasteurizing. Treschow (198) found that needles of Norway spruce, to which $CaCO_3$ was added to increase the pH and which was sterilized for 60 minutes at 60° C., gave a good yield of mushrooms. Recently used other fibrous materials are mixtures of wheat straw and corn fodder (161) or barley straw (46); admixture with wheat straw tends to correct the soggy condition, especially if gypsum is used at the same time. Sinden, in referring to his use of corn fodder, hails it as his "outstanding discovery"; he seems to be entirely

oblivious of the fact that he examined Stoller's thesis (171) in regard to mixtures of corn stalks and wheat straw.

Sources of Nitrogen. In synthetic composts (Table II) Lambert (104) tested many sources of N but obtained best yields with dried blood; better results were had with a mixture of blood and urea than urea alone. In formula shown in Table II, Stoller (177) tested 17 sources of N in a single testing period; 12 of the 17 gave yields of two pounds or more per square foot, with three the yields were one and three-fourth pounds, and with the last two, a yield of one and one-half pounds. The composting period was too long for best results with some of the N sources. The yields with organic nitrogen sources were generally better than those with inorganic sources. Usually $(NH_4)_2SO_4$ is considered unsuitable for preparing composts; indeed, the volatilization of NH_3 from compost containing it prevents even the mycelium from growing. At the same time it was shown that this condition can be corrected by mixing the $(NH_4)_2SO_4$ with ground corn cobs and cheese whey; the yield was then 1.84 pounds. Similar means for suppression of ammonification was applied to urea; before mixing with cheese whey the yield was 0.84 pounds; after, 2.24 pounds per square foot. Subsequent investigators of synthetic composts (161, 41, 44) have completely missed this rather spectacular effect of small amounts of cheese whey (70% lactose) to suppress ammonification. In later experiments (178) the effect of cheese whey and of similar substances has been attributed to a suppression of the "urea" bacteria which produce the ammonification.

Composts prepared with Milorganite (a sewage product), as a source of N, and wheat straw produced only about three-fourth of the yield obtained with brewer grains as a N source. When Milorganite was mixed with spent hemlock bark, no composting was required, except for pasteurizing, and the yield was 2.5 pounds per square foot. Good yields were obtained also with bark and Cyclone Waste (a fibrous Milorganite) and with mycelium of *Penicillium notatum* (178). Aparently there was sufficient tannin in the bark to inhibit the bacteria causing ammonification. Christensen (25) also suggested use of penicillin waste; the % P that it contains is too high and has to be diluted by adding other sources of N.

Sinden (161) attempted the use of calcium cyanamide in mixture of straw and corn fodder; in order to get a relatively satisfactory yield, the amount had to be severely limited. He claims this range as the "optimum amount" and that at higher concentrations it is toxic for an unknown reason. Later Edwards (43) attempted using cyanamide; the composts he prepared with it were a "complete failure", probably not on account of the % N used. Previously it had been shown that under certain conditions cyanamide absorbs moisture and CO_2 and is converted to dicyanodiamide; the latter prevents growth of the mycelium (177).

Edwards (44) claimed that dried blood is a better source of N than the inorganic materials tested. However, he did not attempt to suppress the profuse ammonification of inorganic N by use of cheese whey and ground corn cobs. His poor results with $Ca(NO_3)_2$ probably were caused by the difficulty of microorganisms in the long straw stack to reduce it to ammonia. It has been suggested that $Ca(NO_3)_2$ is most effectively used with fibrous materials which undergo little decomposition (177). In later experiments Edwards found that urea, hoof meal and hide meal were equally as satisfactory as dried blood (43, 46). In his experiments the gypsum used in the compost along with

urea, and the superphosphate added at the end of the composting period, provided, apparently, the acidification to gain a yield of one and three-eighth pounds; the yield might have been higher if cheese whey had been employed.

Mineral Constituents. It seems that an understanding of the mineral requirements in preparing composts for mushroom culture was given an early setback by the publications on this subject by Herbert and Heim (72, 73). Although their publications have been quoted widely, no one attempted to evaluate the accuracy of their analyses or conclusions until it was done by Treschow (197) and Stoller (178), independently. Their analyses are, indeed, fantastic; for example, they find 11% K_2O in the ash of the sporophore, whereas an analysis made in 1867, and which they include in their data, shows it to be 50%; this larger amount compares favorably with recent analysis (60). They admit that the amounts of P and K in their analysis are " notably " lower, but make no attempt to account for the difference. On the basis of the work of a previous analyst, they state that manure is too poor in Ca and K with reference to N and P, and suggest addition of Ca and K. Then, according to their own analysis of manure, they find it too rich in Ca and K in reference to P, and suggest use of phosphoric slag. They conclude that manure contains sufficient N and minerals except Mg and S. An understanding of the mineral nutrition of the mushroom cannot be derived from their confused statements and analyses. The recent statement by Sinden (161) about their priority in the use of K is unjustified.

POTASSIUM. It has been pointed out by Zellner (68), who made extensive analyses of fungi, that the chief difference between green plants and fungi is that the latter contains more K and less Ca. So it is no surprise that addition of K to, say, Richard and Hutchinson's compost should result in an increased production of mushrooms. While there are allusions as far back as 1891 to the use of potash for mushrooms, in none of these has there been shown experimental proof for the requirement of K for sporophore production; such requirement was not established until proven by Stoller (173, 177). By employing spent licorice roots and brewers grains, materials from which the K had been extracted, it was possible to demonstrate an increase of 133% in yield by adding K. It was observed later that sporophores growing on composts with practically no K, still showed only 10% less K than from sporophores on normal composts (183). Sinden (161) confirmed Stoller's results with potash, but his claim to have determined " optimum concentrations " of K is hardly acceptable considering his low yields, his use of Ca cyanamide as source of N, and the fact that he does not appear to be cognizant of the principle of limiting factors. Edwards (39) carried out an experiment with three levels of K, but he found " all yields were so low that the result, though interesting, was of no practical importance " (45).

PHOSPHORUS. As shown in Table II, supplements of P have been employed by nearly all investigators of synthetic composts. In a series of tests with P, K and Ca, Stoller (177) stated that additional superphosphate did not have a distinct effect; however, a re-evaluation of his data shows that there was a 55% increase due to P. The data are reassembled in Table IV. More plots are available as replications, since the small amount of Ca added to some plots, had no effect.

Sinden (161) stated that no increase in yield was obtained by adding inorganic P to his composts. This lack of response to P is explained by his supposition that a sufficient amount of P

TABLE IV

EFFECT OF P_2O_5 AND K_2O ON YIELD (LBS. PER TON LICORICE ROOTS)

K_2O	P_2O_5	Replications	Lbs. per sq. ft.	% increase
None	None	6	0.7	...
None	5	4	1.1	55
21	None	4	1.6	133
21	5	8	1.9	178
Manure control		4	1.7	...

is derived from the grains; it seems, though, that 50% more P was derived from the corn fodder than the grain. Edwards found that by adding superphosphate toward the end of the composting period, the yield was increased 66% (40). With a material like long straw, in which mixing is rather difficult, it should be advantageous to add all the ingredients except the N sources towards the end of composting.

OTHER ELEMENTS. The functions of calcium salts have been discussed. Yields have not been increased by addition of Ca as a carbonate (178, 40), although some Ca in one form or another has usually been present in such experiments. To show the effectiveness of Ca, probably arrangements will have to be made for maximum yields. The elements S and Cl are included in composts as unavoidable constituents of other ingredients. Both are present in the sporophore and probably do affect the yield. It is possible to make certain deductions about the relationship of S to Cl; this will be discussed in regard to the truffle disease.

TRACE ELEMENTS. Physiologically, trace elements are considered nowadays as antagonists to other minerals or as accelerators of prosthetic groups of enzymes. There are some 28 of these elements, so there is the problem of making a selection for the requirements of mushrooms. Stoller (173) suggested the following nine: Mn, Fe, Al, Cr, Cu, B, Zn, Br, I. The basis for selection of some has been made; generally, elements reported as significant in physiology of fungi were included. Each was tested at two levels for toxicity and productivity; no effect was observed except possibly an increase with B, but this was not confirmed. The effect of a mixture of these elements in root composts was variable, but in straw composts the effect was usually significant. The mixture served, besides, as a flocculent and also as a precipitant of source of N in the "coprination" process (173). Edwards (39) adopted the same list of elements and claimed satisfactory increases in yields by including them in the preparation of straw composts. He used a lower concentration of these elements and included another, molybdenum.

VITAMINS. In his experiments in pure culture, Treschow (198) observed that mycelium would not grow on a synthetic nutrient solution unless biotin and thiamin were included. An increase of 35% resulted from adding thiamin to manure compost before spawning; even with this increase, the total yield was only two pounds per square foot. Hoffman (78) claimed a general increase of 15% by spraying beds with thiamin before casing; of the six B vitamins tested, largest increases were obtained with thiamin. He found that if the yields of the controls were high, then the response to vitamins were low, and vice-versa. Lambert and Ayers (107) found no increases in yield with vitamins, even when a composite of 11 were added to the compost in three concentrations at three different times—before, during and after pasteurizing of the manure. Stoller (177) observed no significant increase by adding a stock-feed vitamin concentrate or a fermentation product rich in riboflavin to root composts during composting. Apparently, under the usual conditions of composting, a sufficiency of the B vitamins is synthesized microbiologically.

Supplements to Horse Manure Composts. Lambert and Ayers (107) observed that by supplementing manure with organic N, the yield was increased. There was a linear response in yield to additions of three to seven pounds of N-equivalents, so that for each additional pound of N-equivalent, the yield was increased approximately 25 pounds per ton of manure. Of the N sources tested, only cottonseed meal and brewers grains produced consistent yield responses. No significant increases were obtained by supplements of different levels and combinations of P and K. But no mention is made that for every pound N-equivalent of grains or meal, there was included as part of the composition of the N sources, one-fourth to one-third pound of P as P_2O_5. The effect of the P associated with the N remains to be explored.

About the same time Edwards (43) conducted experiments on fortifying manure with three pounds N-equivalents of $Ca(NO_3)_2$ or dried blood per ton of manure besides K, trace elements and gypsum along with the P, as in his synthetic composts. It might be construed that he obtained the same yield-response to a three pounds-N-equivalent as mentioned above; however, the yield obtained without any supplements was about the same, on basis of pounds per ton, as Lambert and Ayers (108) found by adding seven pounds-N-equivalents. The initial N content of manure is available in only one study (43), so comparisons on this basis are not possible. It appears, though, that Edwards' manure contained more straw, *i.e.*, a larger source of carbon. An explanation on the basis of C:N ratios is discussed later.

Methods of Composting or Preparation of Substrates

Principles of Composting. The purpose of fermenting or composting materials in the preparation of substrates for mushroom culture is, as stated in the previous review (102), to make the substrate unsuitable for competitive microorganisms, yet, at the same time, favorable for growth of the mushroom mycelium and for a large production of sporophores. Waksman and Nissen (205-A) explain that this situation is brought about by microbial growth in which there is a consumption of the water-soluble substances, some hemicelluloses, less cellulose and little of the lignins, so that the latter along with the microbial proteins tend to accumulate. They find that the mushroom does not use the "compost as a whole for its nutrition", but prefers the substances accumulating, namely, the lignins and organic N-complexes. From their data on pure culture it is evident the mycelium can utilize all the fractions of the carbonaceous substrate but prefers lignins. On the contrary, Treschow (198) observed (in pure culture studies on production of dry weight of mycelium) that xylose, a hemicellulose hydrolytic product, was more suitable than other carbon sources. He considered the decrease in hemicellulose during composting a "big minus". The purpose of composting, he reasoned, is not to accumulate lignin but to reduce the large amount of ammonia in fresh manure by converting it partly to microbial protein and by evaporating the rest, so that it would be possible for the mycelium to grow. On the other hand, Stoller found in preparing composts with licorice roots that urea, which is the source of NH_3 in fresh manure, can be utilized by the mycelium without apparent conversion to proteins or evaporation as NH_3, by mixing or "combining" it with lignin or tannin (173, 177, 178). If competing microorganisms could be prevented from interfering, the mycelium could utilize all the constituents of fresh manure; it seems, then, as Treschow (198) has stated, that "composting is no abso-

lutely indispensable requirement for mycelial growth ".

When composting for less than 15 days (105, 177, 163) there is a loss in dry weight of 10% to 35%. The loss is chiefly of carbonaceous material as respired CO_2, and less of N as NH_3. That the loss of C is greater than the loss of N may be estimated from data (205-A, 163) on the composition of manure before and after composting; that is, the C:N ratio decreases as the duration of composting increases. More significant for increased yields than a low expenditure of C and N sources during composting is a high C:N ratio, if it is assumed that the inverse yield-N law is operative. To maintain a high C content in manure, which contains large amounts of urea, it is necessary to add an acidifier, such as gypsum, or to make shallow beds in order to promote oxidative processes (163), or to compost indoors at 115° F. (108-A), etc. The benefit derived from short methods of composting is a low expenditure of C and N sources, especially as a means for attaining a high C:N ratio.

The competitive microorganisms that need be considered are those which are resistant to the temperature of the pasteurizing process. They are:

a) Thermophilic bacteria, actinomycetes and fungi. These are usually considered beneficial and grow abundantly if soluble carbohydrates are available and temperature is over 120° F. (205, 206, 103, 113, 114, 108-A).

b) Ammonifying bacteria. They may be easily isolated in pure culture on a 2% urea broth agar from fresh horse manure (178) and can be suppressed by acids, sugars and starches. The acidifying effect of gypsum and superphosphate may suppress them under some conditions. Cheese whey (lactose, lactic acid), tannin and lignin suppress ammonification (177). Rye flour is probably used for the same reason (21).

c) Acid-favoring molds. The green molds, *Penicillium,* etc., grow on cellulosic materials if the pH is less than 6.0. They are encountered sometimes after sulfur fumigation.

d) Alkaline-favoring fungi. *Coprinus* and *Cheatomium* are most frequently encountered. Conditions favorable for their growth are available cellulose together with alkaline substances or conditions and free ammonia. Holding compost at 100° F. after pasteurizing is favorable to *Coprinus,* whereas pasteurizing at 150° F. or over induces growth of *Chaetomium* (108-A).

e) Mycorrizhal-like fungi. *P. microspora* can grow in the compost only in association with the mushroom mycelium. It will be discussed later.

Rapid Methods of Composting. The first attempt to shorten the duration of composting was made by Stoller, Smith and Brown (186) prior to 1937. A slowly revolving drum was provided with means for forced aeration so that the aeration and temperature could be controlled in order to obtain a rapid, high-temperature fermentation. The naturally high temperatures developing in fresh manure or synthetic compost of cornstalks were controlled by altering the rate of aeration; the temperature was allowed to gradually increase from 95° to a maximum of 145° F. and then back to 104° over seven or ten days. The manure and cornstalks were composted with a loss of dry weight of 20% to 28% in seven days; a normal yield of mushrooms was obtained. While the practicality of this apparatus was questioned, the principle was demonstrated that with better aeration and mixing of the compost, the duration of composting could be shortened.

Later, Lambert (103) in 1938–41 conducted experiments in quart jars and observed that fresh manure could be converted into composts suitable for mycelial growth in about ten days if the

temperature was maintained between 120° and 140°, the moisture controlled by adding superphosphate or gypsum; the rate of aeration was not controlled but assured by diffusion. The loss in dry weight was about 32% in ten days. In 1950 Sinden and Hauser (163) described experiments conducted ten years earlier in two-quart jars with arrangement for aeration at 140° F. Two rates of aeration were attempted, one rate six times as fast as the other. At the lower rate of aeration the loss in organic matter (which is greater than the calculation for loss in dry weight) was 19%; at the higher rate, 37%. Growth of mushroom mycelium was poor unless compost was extracted with water.

One of the factors of importance in "short methods" is the moisture content of the compost. Composting, or the decomposition of plant materials, is essentially a hydrolytic process; also, with better aeration, more moisture is required to replace losses. With licorice root composts, the problem of moisture is not apparent because the roots as received from the manufacturer of licorice contain about 220% moisture, and even after pasteurizing they retain 200%. For manure with its straw bedding, the question of moisture at start of compost-

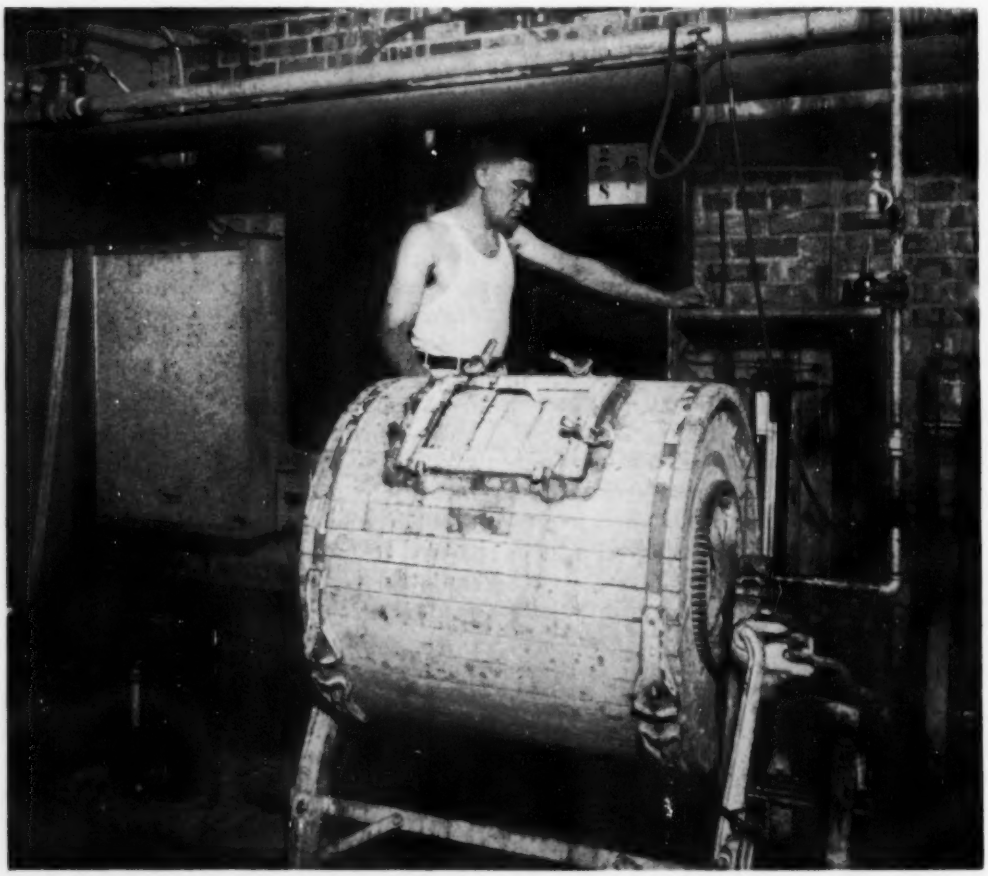

FIG. 1. Apparatus for rapid, thermophilic, aerobic decomposition of cellulosic materials in preparing composts (186).

ing is of paramount importance. It is to Lambert's credit that he was clearly cognizant of this fact and suggested increasing the moisture to 250% at the start of composting. Upon publication of Pizer's information on the flocculation and water-controlling effect of gypsum (139), Lambert (105) applied this information to increase the moisture content of manure composts, so that the duration of composting could be shortened and the quantity of manure per bed area extended. The usefulness of gypsum, it seems to the writer, is not only to control absorption of moisture but also to help suppress urea bacteria by its acidifying effect. In his experiments Lambert found that by mixing superphosphate or gypsum with fresh manure, preferably chopped, adding water to 250% moisture and filling the beds immediately, the fermentation could be reduced to 12 days, the bed area filled to 200 square feet per ton of manure, and the yield was 1.75 pounds per square foot or 350 pounds per ton. The difficulties with his method were that watering and mixing had to be done in the beds, and the temperature had to be lowered and raised again to do it. However, he clearly realized that in commercial practise, the outdoor composting could be " reduced to 2 or 3 turnings at rapid intervals " so that the necessity for working in the beds could be eliminated.

The next important factor in rapid method of composting is controlling the aeration and temperature of the compost heap. A drum, as described above, would be ideal if an apparatus of this kind were practical. But in the compost heap, as stated by Lambert (103), " the physical conditions (temperature, moisture, and aeration) are dependent largely on the size, shape and compactness of the heap ", although he did not give an experimental demonstration of these dependencies. Stoller (177), in his experiments with licorice roots, described experiments on the principle of the relation of size of pile to aeration, duration of composting and effect on yield. With small piles yields of 2.5 pounds per square foot were obtained, but with large piles, even though the base was only nine foot and height four to five foot, the yield was reduced to 1.5 pounds. It was evident that with small piles the surface exposure was relatively large and the " core " of the pile was relatively small so that good aeration was obtained; for large piles, the conditions were the reverse. This condition of the large piles was corrected by placing a " ventilator " in the center at the bottom of the pile (use of ventilators under compost heaps is a well known practise). Diagrams and conditions in the piles (moisture, pH, temp., CO_2 and O_2) with and without ventilators were illustrated.

Experiments on the effect of the duration of composting on the yield were devised with small piles of licorice root composts (about $\frac{1}{2}$ ton); that is, under conditions of good aeration. A yield of 2.85 pounds per square foot was obtained after composting for eight days and turning the compost twice. The yield was decreased to 2.55 pounds by composting 16 days and three turnings; by composting only two days and one turning, the yield was reduced to 1.5 pounds and all kinds of mold growth encouraged. It is possible that with better means for mixing and aerating, the duration of composting, at least with roots, can be reduced to less than eight days and large yields still obtained. But with large piles it was stated (177) that proper mixing, watering and aeration were not so easily managed, so that 15 to 20 days were required for composting. However, with the recent advent of the tractor-loader and the newer automatic self-feeding loaders and turners, mixing and aeration can be accomplished as

effectively in large piles as in small ones, so that the duration of composting, at least with roots, would not need to be longer than eight days.

The "short composting system" described by Sinden and Hauser (163) pertains to the two "important objects": (*a*) bringing up the moisture at the beginning of composting to 250% and then adding supplements such as gypsum or protein, and (*b*) facilitating aeration of compost and preventing the anaerobic "core" by keeping the cross-section of the pile small. This "system" is not fundamentally different from the process described by Stoller (177) above with licorice root composts; except, employing manure instead of roots, they adopted Lambert's method (105) described above, pertaining to an initial moisture of 250% and the use of gypsum. Judging by the two "important objects" of their "system", it is not apparent to the writer where they have introduced any new principles in the short method of composting; their system may be characterized as a "variation" of Lambert's procedure. The fact that Sinden and Hauser (163) made no reference to previous publications on mushroom research, is probably contributory to Sinden's being erroneously referred to by some writers and growers as the originator of the "short method" of composting. But it does seem to the writer that Sinden and Hauser have made a notable contribution in their last experiment which was briefly reported on the production of 800 pounds of mushrooms per ton of manure; this experiment will be discussed later.

FIG. 2. Automatic self-feeding compost-turning machine. Turns compost at rate of 60 tons per hour.

High Carbon Content of the Composts. From laboratory-scale experiments on composting (186, 103, 163) it

is evident that the loss in dry weight is dependent not only on duration of composting but also on the temperature and rate of aeration. With outdoor composting only the duration can be completely controlled. Arranging the size of the pile will tend to reduce variations due to the effects of temperature and aeration but will not eliminate them. So it is not surprizing that in one set of data reported by Sinden and Hauser (163) there is a gradual decrease in bed space filled as the duration of composting is increased from seven to 27 days, whereas in another set of data there is no such clear distinction. In order to account for their yields of 700 to 800 pounds per ton, or yields recently reported by Lambert and Ayers (108-A) of over 500 pounds per ton, the percent loss of dry weight would necessarily have to be low. Sinden and Hauser's method to counteract the effects of ammoniation and susceptibility of the compost to *Chaetomium* is to make loose shallow beds, assumed to be about 400 square feet per ton according to their data of an average of two pounds per square foot or 800 pounds per ton. Lambert and Ayers make deeper beds (73 to 146 square feet per ton) and allow the NH_3 to be dispelled, more or less, by holding the temperature at about 115° F. for five days; in order to arrange for longer cropping period, the beds are initially heated at 150° F. to destroy pests. The principle, however, is the same—a high carbon content of the compost. Previously the amount of fixed carbon in the compost was assumed (*i.e.*, taken for granted without testing) to be more than sufficient, and the function of the fibrous material was considered chiefly to provide an aerobic substrate. The significance of high carbon in the compost was foreshadowed by Treschow (197), predicted by Sinden (161) and empirically but not functionally shown in experiments by Sinden and Hauser (163).

A high moisture content is required with a high carbon compost (163, 108-A), and the yield is increased with a higher percentage of moisture (108-A); however, the licorice roots had 66% more moisture (200% on basis of water present), and the maximum yield was 360 pounds per ton (177). It might be judged from Lambert and Ayer's experiments that, had there been better eradication of insects, the yields with the root composts might have been greater. On the other hand it was shown that the highest yields with the roots were obtained when organic N materials " served also as a source of carbon " (177). With inorganic N substances it was suggested to add " very finely ground straw or corn cobs " besides cheese whey (177). The higher yields with organic N would indicate, then, that the roots were deficient in available carbon. Actually licorice roots have less readily available carbon; for example, the content of hemicelluloses in licorice roots is one-third that of straw, one-half that of manure. In addition, roots have twice as much lignin as straw or fresh manure. So it seems that the more available carbon—the celluloses—is the basic critical element involved. This factor was not appreciated (" unpremeditated "), when conducting experiments with licorice roots.

K : N Ratio of Compost. In order for the high C:N ratio and the inverse yield-N law, previously mentioned, to operate, the N:K ratio should be low. It has been shown rather conclusively with green plants (200) that N and K are inversely related. To apply this information to mushroom culture it would be required to greatly increase the amount of K as the N is diluted by a large amount of C. In other words, the explanation for the previous investigators' (108-A, 163) obtaining such high yields with reduced composting of manure lies not only in a high C:N ratio but also in the fact (fortunate for them)

that manure contains a large amount of K (see Table II).

Some evidence for an inverse K:N ratio in mushrooms was found in the analysis of mushrooms grown on a K-deficient compost (183). Mushrooms analyzing low in K were high in N and P, and conversely. Instead of depending upon the inverse relationship of %N to yield (*i.e.*, according to inverse yield-N law) in order to determine the yield potentiality of a compost, it may be more useful to determine the K:N ratio of the sporophore. For example, if the %K is found relatively high and the %N low, the yield may be expected to be comparatively high. This procedure may be more accurate than merely comparing the %N of sporophores from different composts, since the %K and %N is determined in the same mushroom or sporophore specimen.

Other Methods of Composting. Besides preparing composts with gypsum to obtain rapid fermentation, other methods and procedures have been described. One of the earliest attempts at synthetic composts was the process described by Szuecs (190) in which the ingredients (peat, molasses, $(NH_4)_2SO_4$, superphosphate) were acidified to pH 2.0 in order to bring about the predominance of mold growth. The fermentation with *Aspergillus* lasted two to five days, and temperature rose to 158° F. Thereafter the substrate was sterilized and inoculated with spawn. This process is not known to be practised in the U.S.

"Since one of the chief functions of composting is to make the N unavailable to most microorganisms by its combination with lignin" (177), Stoller proposed steeping N sources in lignin, a by-product of the paper industry, besides other protein coagulants, such as are used in tanning—tannin, chrome, etc. Several N sources were tested and found satisfactory. Even urea, as has been mentioned, could be used without composting, except for the short period of pasteurizing. The procedure consisted in adjusting solutions of these "coprinating" agents to pH 5–6, steeping the N sources for several hours, then mixing with minerals and absorbing the whole mixture in roots, barks, etc. The yields varied with N sources; with urea the yield was 2.25 pounds per square foot. Where these materials are available, this method has good possibilities.

Recently Carr (20) revealed some of the "secrets" of the "docket system". Fibrous materials, *e.g.*, cardboard box paper, is soaked in solutions of various "trace" elements, including chrome alum. Inulin serves as an additional source of C, and dried protozoan bodies are applied as "mere covering". The mushrooms are formed directly on cardboard substrate. It appears that the lignin of the cardboard, besides chrome alum and trace elements, suppresses microbes after sterilization. Inulin has been mentioned in several connections as tending to stimulate sporulation or fruiting (102, 13).

On account of the unstable climate of Denmark, Christensen (25) developed a procedure for composting within an enclosed room. The temperature of the compost heap is regulated by altering its size. When insects are brought to the surface by the internal heat of the heap, the room is disinfected by burning S; in this way the "sweating out process" is avoided. Composts are fermented and ready for spawning after 16 to 28 days, depending on outside temperatures.

A procedure comprising an improvement over Christensen's method and incorporating the advantages of mixing, agitating and aerating, as exemplified in submerged culture, has been developed by Stoller (180). Actually the method represents a further development of the apparatus employed by Stoller, Smith and Brown (186). With this apparatus

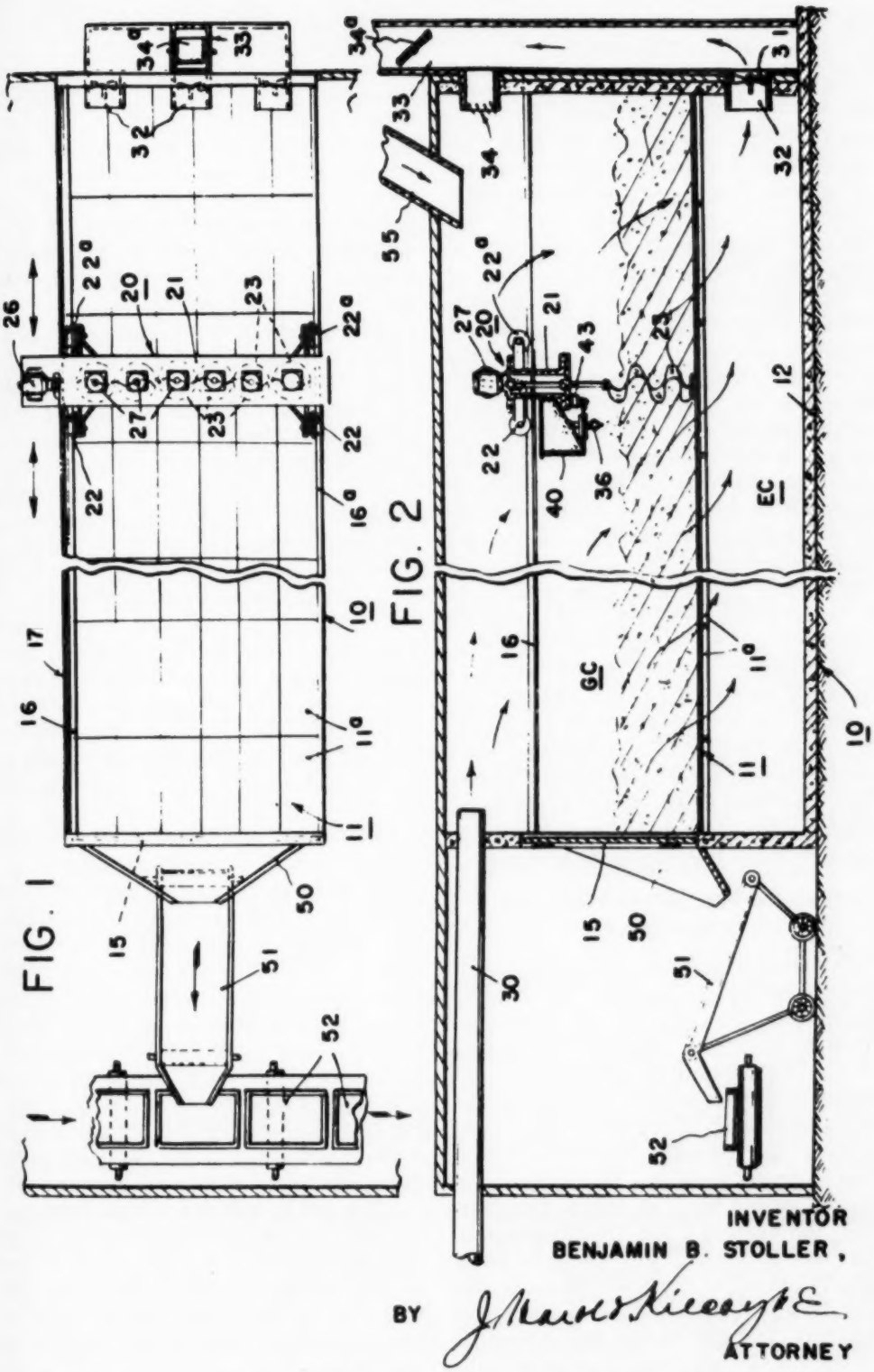

FIG. 3. Apparatus for a completely mechanized automatic system of composting and spawning. Arrows show direction of air currents; No. 21 is turning machine, No. 11 is perforated floor. Pat. Pend. (180).

(Fig. 1), which consisted of a "butter churn" or slowly revolving drum, as mentioned previously, the principle of a rapid, aerobic thermophilic fermentation of composts for mushroom culture under controlled conditions was established. All that was necessary to be done was to reduce the process to practise.

The apparatus (Fig. 3) and procedure are relatively simple. The idea of a carriage moving on a rack and pinion has been adopted universally in such machines as lathe, meat cutter, typewriter and saw mill. All that was necessary to modify this apparatus for mushroom culture was to suspend suitable screws with large blades from the beam of the carriage, and provide a perforated floor for aeration. Air is drawn through the mass of material as it is turned, under controlled conditions of moisture and temperature, so that the duration of composting is reduced to the few days formerly constituting the pasteurizing process. This method affords the possibility for holding C and N losses to a minimum and for complete standardization. It has many other advantages, although the large initial capital investment required may delay its adoption.

Factors Affecting Development of Sporophores

Problems Involved. Since the seventeenth century it has been the practise to induce development of mushroom fruiting bodies by covering the surface of the mycelium-impregnated compost with a layer of loam about one inch deep. The function of this layer of soil in initiating fructification has never been proved experimentally. It has been suggested, on the basis of Klebs' principle, that the tendency for fructification in the soil is caused by the soil being largely devoid of nutrients yet favorable in its reaction, temperature, aeration and moisture for growth of the mycelium (102). This principle has been propounded in the statement that "vegetative vigor was antagonistic or opposed to sexual reproduction" (168). This reciprocal correlation has been explained further on the basis that the ratio of thiamin to carbohydrates is greater in dilute solutions, or soils, than in concentrated media, so there is sufficient thiamin to influence fructification (69, 196). The effect of thiamin is to increase the rate of respiration (70). Evidence for larger production of mushrooms by use of thiamin, as previously discussed, has not been conclusive. Allusions have been made to the employment of an unfavorable carbon source, such as inulin, to induce fructification (102, 13, 20). In the case of the mushroom mycelium, swellings of the hyphae, like tubers which were found to be filled with glycogen, have been observed (154). So it may be inferred that reserve food has been stored for reproductive purposes. Others have obtained fructification of fungi on moderately concentrated nutrients (168). But the mechanism for fruiting is not indicated on the basis of a dilute substrate being favorable to fructification.

Production of sporophores depends upon "special nutrient requirements" (102) only in so far as such requirements affect the magnitude of the yield. The multiple of the NPK elements, as well as the C:N ratio, etc., will determine the size of the crop, provided the casing medium is in an optimum condition for transmitting the nutritive capacity. Although mushrooms will form where water from condensation on a ceiling hits the bed, neither spraying beds without soil or applying moist vermiculate will induce a normal crop. Even heating soil to relatively high temperatures will prevent normal function of the soil casing (109). A study of the effect of heat on soil, in which factors are introduced to overcome the toxicity of heat, may shed light on the function of the casing.

The contradictory nature of the soil

problem is that the problem seems so simple, yet does not lend itself to experimental demonstration. In a recent comprehensive discussion by the writer (182, 183, 184), about the function of the soil, many relations of the soil to fructification were discussed, but no explicit proof of its function was provided. The reciprocal relationships of the vegetative and reproductive influences are easily observed with mushrooms. For example, the mycelium tends to grow through the soil, forming a dense white mat or "stroma" (186-A) without any sporophores if the moisture capacity of the soil or the air movement is insufficient; this condition is more easily demonstrated with peat (184). Under limited conditions of aeration the air has to be filtered through the soil to induce fruiting; otherwise only mycelium grows on the surface (178). Only when a suitable cropping soil is placed above a poor one, and not in the reverse, was good production possible (142). So it appears that in order for fructification to be initiated, the mycelium must be in close contact with the soil particles, in the presence of sufficient moisture and at the junction with oxygen. The question as to what happens at the point of contact of soil and hyphae may be discussed more advantageously after considering other factors affecting sporophore development.

Characteristics of Casing Soil. The yields of mushrooms from beds cased with silty or clay loams were found to be much higher than from sandy loams and sand (109, 142). Non-fibrous peat (*e.g.*, humus) mixed with limestone and gypsum made an excellent casing, but fibrous peat favored rapid mycelial growth through it without producing mushrooms (184). Clay loams and peat are highly colloidal materials with immense surfaces and high cation exchange capacity. The suitability of a casing may be determined on the basis of its exchange capacity. The fact that a larger yield was obtained with peat (184) than with a soil (109) of the same natural reaction (pH 4.8–5.0) points to the effect of the greater exchange capacity of the peat. The water-holding capacity of soil is considered an important factor, and a mixture of vermiculate and peat (fibrous?) with 28% moisture resulted in the best yields (10). But non-fibrous peat (hyperhumus) with 65% moisture (109) and similar peat with 70% to 80% moisture (184) also were highly productive. It appears that yields will be higher for soils with better water-holding capacity, provided aeration is sufficient. Flegg (55) suggested that the optimum moisture range of a casing be determined by its moisture equivalent. He found the mean moisture content of casing soil on beds to vary from 11% just before watering to 20% just after watering.

"Calcium soils", that is, soils in which the Ca ions have been exchanged for H ions, are preferable for casing beds because they favor high moisture retaining capacity and good aeration. $CaCO_3$ is preferable for neutralizing soils (109). When the soil reaction was reduced to pH 5.2 by adding H_2SO_4, the yield was barely affected, but when the pH was reduced still further, the yield was reduced (109). The fact that a good yield was obtained with a soil at pH 5.2 by adding H ions may be attributed to the crumb structure not having been destroyed. Similarly the fact that yields of mushrooms were about the same from soils varying in pH from 5.5 to 8.5 (109) may indicate that the crumb structure was equally good. But structures of soil stabilized even by Ca can be destroyed by purely mechanical means, such as watering. The mycelium of fungi has been found especially effective in stabilizing soil structure (122, 61). In fact, Edwards and Flegg (49) recommend that watering of beds be commenced after the mushrooms mycelium is grown into the soil to prevent

destruction of soil aggregates. They found that spraying water in small amounts at three- or four-day intervals inflicts less breakdown of soil structure than heavy watering at longer intervals.

With regard to cultural practices relative to casing, it was found that thickness of soil layer was not an important factor; the customary one-inch layer seemed preferable (109). Two or three weeks after spawning seemed to be the best time for casing (109, 40). The need for lowering the temperature to induce fructification has been interpreted as a means for raising the humidity of the atmosphere (10); however, it seems more likely that the effect of lowering the temperature is to provide for increases in the solubility of atmospheric gases, in the rate of air flow (by diminishing the viscosity) and in pH (183). The need for "thermal induction" to induce reproductive development in certain plants (beets, celery) is well known. Stout (187) explained the effect of this lowering of temperature to induce reproduction in beets as decreasing the oxidation-reduction potentials. Courtieu (29) observed that organic N of soil should be between 0.007 and 0.018%; too much N stimulates the development of too many mushrooms, all of which do not develop to maturity. He finds that the pH must be between 7.2 and 8.2 for a good yield and that soil should contain 2.5% to 3.0% active Ca.

Relation of CO_2 and Atmospheric Conditions. The stems of mushrooms growing on beds sometimes elongate abnormally, and at the same time growth of the caps is retarded. Lambert (100) claims to have demonstrated experimentally that this abnormal growth is due to the injurious effects of CO_2 released during respiration of sporophores. An evaluation of his results reveals, however, that there is a slight, if any, elongation of the stem when 0.2% to 2.6% CO_2 introduced under the bell jars is derived from commercial cylinders, whereas severe abnormal growth occurs if the gases (1.8% CO_2) accumulate naturally from respiration (see his Fig. 5, A to F compared to G). Stoller (171) called Lambert's attention to this discrepancy; the latter (101) acknowledged the difference but suggested that it might be attributable to the greater susceptibility to CO_2 of mushrooms from older beds. The writer (171, 178) has observed the same effect of CO_2 from respiration on both old and new mushroom beds many times, so Lambert's explanation is probably incorrect; besides, abnormal growth may occur even when the CO_2 is absorbed in KOH (171). Growth was stunted or killed by introducing 6% to 20% CO_2 under the bell jars (100).

Stoller (177, 182) has shown that no CO_2 accumulated in bell jars with two openings, one at the top and the other in the side at the bottom, and fastened over mushrooms growing in pots; despite the complete absence of CO_2 there was distinct elongation of the stem and retardation of the cap. The mushrooms growing under bell jars with an opening only in the top of the jar, so that CO_2 accumulated therein, were severely abnormal. But the abnormal growth of the mushroom was much more marked where the CO_2 accumulated naturally than where the CO_2, at the same concentration, had been introduced under the jars from commercial cylinders, as in Lambert's experiments. Accordingly it is assumed that an unknown volatile substance was present in the CO_2 accumulating naturally, and that this substance, not CO_2, is responsible for the abnormal growth.

Lambert (100) inferred that the CO_2 accumulating when jars are inverted over mushrooms growing on beds is liberated by the sporophores because he observed that only 0.2% CO_2 was present under jars after five days when placed on the beds where mushrooms had grown. Stoller (171) observed that

the CO_2 accumulating in both the compost and soil, and under bell jars, depended upon watering the soil; later (178) with better control of the physical condition of compost and soil, it was observed that before watering, the CO_2 in the compost-soil atmosphere is 0.2% to 0.6%; after watering there is a rapid rise in CO_2 to 1% to 2% in about 24 hours, then recession gradually over a period of eight days. Burrows (17) showed that both the sporophore and the compost-soil respire considerable CO_2; but it is not possible to correlate his data with conditions in the bed.

Mader (119) observed in caves where air circulation or "drainage" is at a minimum and CO_2 concentration negligible, that sporophores failed either to develop or to mature. He claimed to have demonstrated experimentally the removal of toxic substances inhibiting fructification by growing mushrooms to maturity in "air-tight chambers" of 12 cubic feet; in order to do this the atmospheric gases were purified and recirculated by pumping them through air-washing equipment containing mineral oil, alkaline permanganate solution or activated charcoal. But if the atmospheric gases are circulated through alkaline permanganate solution, the CO_2 resulting from metabolic processes would be absorbed, the oxygen would be consumed and a partial vacuum would result. Mader mentions neither any analysis for CO_2 in these experiments, nor the presence of a partial vacuum, nor introducing additional oxygen. It is not comprehensible how fruiting bodies of mushrooms could be initiated and develop normally in the absence of oxygen. A more complete criticism of his procedure is published elsewhere (182). It is highly improbable that his equipment was "air-tight". His experiments demonstrate only induction of fructification by increased air-drainage or ventilation. The evidence was insufficient to prove that a volatile toxic substance that prevents fructification had been removed.

Stoller (178) attempted experiments similar to Mader's but grew the mushrooms in glazed pots fastened to bell jars with rubber bands. The air was washed in alkaline solution and recirculated by rubber bulbs actuated mechanically (Fig. 4). The partial vacuum resulting from absorption of CO_2 and consumption of O_2 was evident not only by reading a manometer in the train but also by the indentation of the rubber bulbs caused by suction. This condition was corrected by systematically admitting pure oxygen. Even so, only mycelium grew over the soil and no sporophores developed to maturity. Mature sporophores, however, were obtained by an arrangement to filter the air through the soil after it was washed in alkaline solution. Mushrooms developed only in the locality where the air filtered through the soil. Unfortunately no provision was made for a column of soil in the gas train for filtering the air, so that it is uncertain whether a volatile substance was removed or whether the air has some more direct effect on the mycelium or upon enzymes diffusing from it.

Attempts have been made to observe effects of ventilation under experimental conditions (178) and in mushroom houses (127, 186-A). In preliminary experiments with arrangements of pots and bell jars as described above, it was observed that the stems were elongated and caps retarded when the air current was 0.11 liter per minute; when the rate was doubled, the mushrooms grew normally. But when the mushrooms were growing three times as numerously, the stems elongated, even though the current was increased three to five times. The CO_2 in the bell jars was 0.2% to 0.4%, yet the stems elongated, with the one exception mentioned (178). In observations on the relationship of yield to free air space in mushroom houses, it

was shown that as the ratio of the volume of air in the house to bed area was increased, there was an increase in yield of mushrooms and a more rapid production. The optimum volume of air required is about six cubic feet (127) to four cubic feet (186-A) per square foot of bed space. This amount could be reduced by use of forced-air ventilation (127). It is suggested that the air in mushroom houses during cropping be changed twice an hour (186-A).

with a moving one". Data are presented showing the difference between evaporation by diffusion and in an air current. The mushroom is regarded as comparable to a "living atmometer". The effect of relative humidity and air-movement could be determined as the "relative transpiration" (ratio of rate of evaporation of sporophore to that of atmometer). In fact, it is still questionable whether elongation of the stem and retardation of the cap, explained as

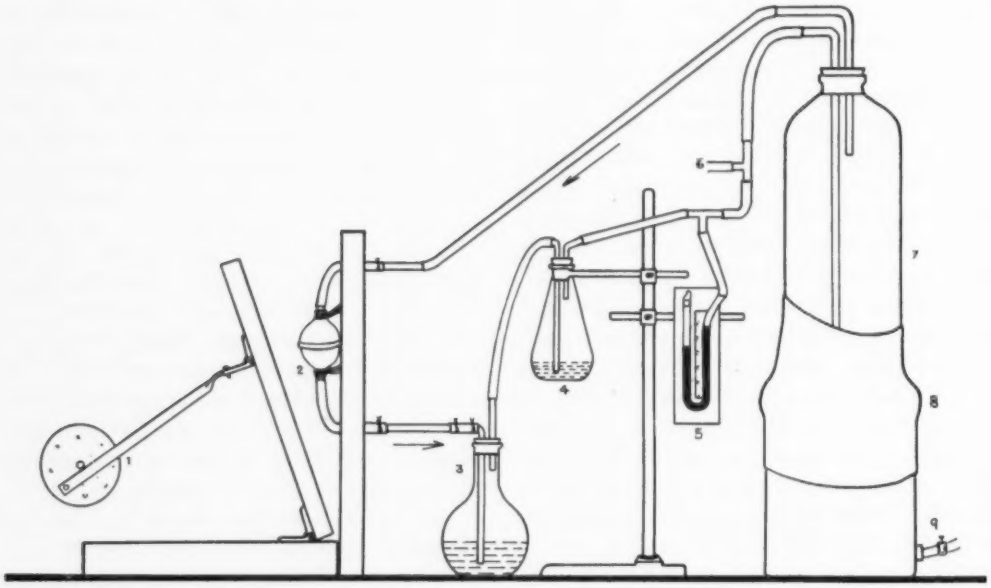

FIG. 4. Apparatus for studying effect of aeration on sporophore development. Nos.: 1—mechanism for actuating rubber bulb pump, 2; 3—KOH soln.; 4—H_2O; 5—manometer; 6—inlet for O_2; 7—bell jar fastened by rubber band, 8, to glazed pot, 9 (178).

It is well known that mushrooms grow normally at high relative humidity, provided the volume of air and air movement are sufficient. But Lambert's surmise (102) of Stoller's experiments is that "high humidity may cause a deformed growth of mushrooms". Stoller (171), however, referred specifically to the effect of humidity only as it occurs "in a still, confined atmosphere" as provided under a bell jar. In fact he states that "the effects of humidity in a still, confined atmosphere contrast sharply

effects of CO_2 or other volatile substance, may not be related to the rate of evaporation of moisture from the sporophore.

Mechanism of Sporophore Development. A working hypothesis may be contrived that a volatile substance formed by the mycelium may affect the initiation and development of sporophores in the soil. A volatile substance of an oxidizing nature emitted by the mycelium has been detected by indicators (*e.g.*, benzidine) suspended over

the mycelium-impregnated compost (175). Furthermore, in some experiments conducted by the writer in 1940 (185) it was observed that tomato plants showed signs of "epinasty" when the young potted plants were inserted either under bell jars on beds where mushrooms were growing or in 3-gallon bottles of spawn; thus, it may be inferred that a volatile, unsaturated substance evolved from the mushrooms and spawn. Whether or not this volatile substance is pertinent to the problem of fructification is unknown; if it were considered in the nature of a peroxide or a quinone (175), it fits into the hypothesis. Quinones and peroxides are known to be volatile at ordinary temperatures and are strong oxidizing agents. Their formation by the enzymatic activity of the mycelium has been reported (63). Lignin could be a source of dihydroxy benzenes, such as catechol (167), which can be oxidized to quinones. The seeming high oxidation potentials of substances diffusing from the mycelium (174) are further evidence that quinones are involved. The necessity to adjust the soil at a relatively high pH, to practically filter the air through the soil (178) and to arrange for the satisfactory cropping soil to be in contact with the air (142) tend to show that the medium must be suitable for rapid oxidation-reduction reactions. The decrease in the oxidation-reduction potential that follows "thermal induction" (lower temperature) is regarded as the basis for the induction of reproductive development in beets (187); lowering the temperature to induce fructification of mushrooms may have a similar effect on the oxidation-reduction potential of the mycelium. It appears that the oxidizing intensity of these substances is sufficient to prevent the thickenings at the ends of the strands of hyphae from which the sporophores develop. Accordingly at least one of the functions of the casing soil is to provide an alkaline-oxygenated medium for destruction of this volatile substance (181, 182).

Another interpretation may be had from the recent research of Lindeberg (116-A) in which it is shown that there is a transition in enzyme activity from the mycelium to the rhizomorphs in the casing soil and then to the sporophore. He observed that the mycelium produced a "laccase", the sporophore, a "tyrosinase" and the rhizomorphs a mixture of both. It is possible that fructification is induced by such a transition of enzyme activity. But the existing knowledge is insufficient to indicate whether the oxidation-reduction reactions occurring in the casing soil are mediated by direct enzyme activity or by secondary volatile products.

Diseases of Sporophore and Mycelium

Fungicides. Fumigation of casing soil with chloropicrin (93, 3) was found effective in the eradication of *Mycogone*, *Verticillium* and *Dactylium* diseases. Ammonia at the rate of 0.15 gram per liter of air, introduced in a fine vapor under pressure for six hours, was effectively used as a fumigant in empty mushroom houses; germination of spores of fungus causing the truffle disease was retarded four months or longer—long enough to harvest a crop (176). Spores and cultures of *Mycogone* and *Verticillium* exposed to ammonia at the same time were also killed (185). Wood treated with the following preservatives had no adverse effect on mycelial growth or on cropping: 5% $CuSO_4$, Cuprinol, Celcure solution and 2% Triolith (141). Derivatives of dithiocarbamic acids most effective against *Mycogone*, *Verticillium* and *Dactylium* have been zinc-ethylene-bisdithiocarbamate (Zineb) (217) and tetramethyl thiuram disulfide (TMTD) (38). Several sulfur fungicides increased the yield 20% when sprayed on beds after spawning and before casing;

these are derivatives of dinaphthylmethane-disulphonic acid (79) and disodium ethylene bisdithiocarbamate (184). According to the implications in the contention that the soil acts as a reducing medium, S compounds should enhance this property. Antibiotics derived from microorganisms have been shown to be active against *Mycogone*, but were uneconomical (152). Nevertheless it has been suggested to use the antagonistic microorganisms themselves to control damping-off fungi (66). Spotting of mushrooms was held in check by spraying with a solution of one milligram of thiamin and 0.5 milligram Na metasulfite in 20 liters of water (152).

New Diseases. Of the new diseases described since the previous review (102), the "mummy" and the "mat" or "vert-de-gris" seem most serious.

The "mummy" disease was first recognized in 1935 in Missouri (199) and in 1941 in Pennsylvania (98). Tucker and Routien (199) have made an extensive study of this disease. It is identified at first by the appearance of smaller mushrooms and by slower growth, later by various distortions of sporophores caused by secondary invasion by microbes. Other striking symptoms are development of rhizomorphs in the casing and compost, and difficulty in pulling up sporophores. The disease spreads from the site of infection in the bed at the rate of a foot per day. It has been transmitted only by infected soil or compost, not sporophores or insects. Peak-heating, severe freezing and various chemicals destroy it. The causal agent has not been identified; its type of transmission suggests that it may be a virus. Kligman and Penny (98) claimed that by allowing the soil to dry and then spraying with 2% formaldehyde, all mushrooms appearing thereafter were normal. On the contrary, Tucker and Routien (199) found that not only was chemical treatment ineffectual, but even after recasing the disease persisted. The infection occurs in compost as well as in soil. An outbreak of this disease in Switzerland, possibly the first in Europe, has been described recently (57).

The distinguishing symptom of the "mat" disease is a mat formation, just beneath the casing soil, that prevents sporophore development. The causal organism has been tentatively identified as a fungus, *Myceliopthora lutea*. Means for controlling it are unknown; peak heat of 140° F. does not completely eradicate it (120).

A disease referred to as the "La France" cannot as yet be characterized from its published symptoms except that it is similar to the mummy disease (164). Another, called the "Vern Astley", has for its causal fungus a species of the genus *Spicaria*. It infects the compost and mushrooms, resulting in distortions of sporophores and in reduced yields (164). The last two diseases may be eliminated by the usual sanitation. A dense white mycelium growing through the compost and on the surface has been tentatively identified as *Corticium centrifugum* (7). Its effects were not described.

Further Studies on Known Diseases. Damping off, in which mushrooms become dry and rubbery and degenerate into small unsalable specimens, has been associated with the presence, in the casing soil, of *Fusarium* (chiefly *F. oxysporum* and *F. martin*) (214). Wood (215) noted also that the mushroom mycelium is intensely antagonistic to *Fusarium* spp. It can be controlled by the usual soil sterilization. Kligman and Penny (98), however, found that casing soil infected with similar species of *Fusarium* did not cause injury to mushrooms as decribed by Wood. It is notable, though, that Wood isolated *Fusarium* spp. from the casing soil, whereas the others obtained their *Fusarium* from "infected pine seedlings". It is known that strains can arise from adaptations

to specific substrates; Wood's species may be this kind of strain which appears to be inflicting considerable damage in England.

Further discussions of other known diseases concern a new species of *Verticillium* (197, 5), characteristics of *Dactylium* as a facultative parasite (53), proof that *Monilia fimicola* is a true parasite (54), *Xylaria vaporaria* (216), and evidence that "bacterial pit" pertains to holes in mushrooms eaten out by mites, bacteria merely producing secondary infection (98). This latter view was not confirmed (38), and it has also been suggested that the pits may be formed by nematodes (195-A).

A significant contribution by Yoder, Sinden and Hauser (217) pertains to the use of zineb for the control of pathogens infesting the casing soil. They claim that zineb can reduce or almost eliminate infection after it has appeared, but recommend application after the first watering. One case where a larger yield was obtained by steaming the soil than by dusting with zineb, they attribute to destruction of nematodes by the steaming. Another explanation, however, is possible from Duncan's experiments (38): control with zineb was much better on soil artificially infected on the surface than with soil which was naturally infected throughout because the spores distributed deeper in the soil were not reached by the fungicide. Similarly, in the case above, dusting killed spores only on or near the surface, whereas steaming killed all the spores. Duncan (38) found that TMTD was as effective as zineb. TMTD, under the trade names Arasan, Thiosan and Tersan, has been advertised on the back cover of "Phytopathology" for many years, and possibly tested. The tests failed, probably, because spores were present throughout soil or the fungicides were applied too late.

Truffle Disease and Mycorrhizal Association. *Pseudobalsamia microspora* Diehl and Lambert, the pathogen responsible for the disease in which fructification of the sporophore of *A. campestris* is prevented, is a very interesting organism. Its spores are remarkably resistant to high temperatures and chemicals. It has been studied extensively, prior to 1938 as well as later (62, 175, 176, 177, 96, 37, 38). It is generally considered a "weed mold" competing with the mushroom for the substrate, although the writer is inclined to regard it more of a parasite because it can grow only when associated with the mushroom. In regard to parasitism, authorities state: "The boundary between saprophytism and parasitism is blurred. There are all gradations from partial to complete saprophytism and parasitism" (125). One of the strong proponents for the "weed" theory is Kligman who writes: "Good evidence that the truffle is not parasitic on mushroom mycelium is afforded by the fact that it will not grow on a rye grain medium" (96). If all it takes to convince contenders of the weed theory is to show how the truffle will grow on grain medium and on a grain medium overgrown with spawn, the task is easy. Merely add one gram of $MnSO_4$ to the usual amount of grain in preparing a bottle of grain spawn, and the truffle will grow and fructify profusely (185). But adding $MnSO_4$ to non-sterile compost does not induce the truffle to grow, unless associated with the mushroom mycelium. The order of effectiveness of trace elements in influencing growth on grain is as follows (185): $Mn > Fe > Zn > Cr > Co > Cu$. The Cu usually prevented growth of truffle, even when mixed with Mn. Media preferable to grain in studying this association consist of corn cobs, previously described, to which various N sources, such as dried blood, NH_4NO_3, $Ca(NO_3)_2$ and gelatin, are added; the truffle fails to grow in association with the mycelium unless, in preparing these media, other substances, for

example, $MnSO_4$, gypsum, superphosphate and H_2SO_4 are included (185). A brief résumé of some of the writer's research on the truffle disease has appeared as a contribution of the L. F. Lambert Laboratories (111). The requirements of Mn and S for truffle growth may be to supply methionine synthesized by the mushroom, since Mn induces this synthesis (159); or it may be related to enzyme activity (36), oxidation potentials (91), etc.

P. microspora might be considered as being in a mycorrhizal association with *A. campestris*. Another species of this genus, *P. magnata*, is known to grow in wet clay under oaks and pines (137). Pines, as is well known, are the mycorrhizal plants par excellence (90). Lambert (102) has mentioned that the "true" truffle grows in clay soils associated with roots of oaks, and also remarks on "an edible desert truffle associated with (possibly parasitic on) the roots of *Helianthemum* spp.". It is possible to make certain deductions about mycorrhizal fungi in regard to their content of SO_3 and Cl from Friese's analyses (60). Mycorrhizal fungi (true truffle, *Boletus*, morel, *Lactarius*) except *Amanita*, have more SO_3 and less Cl, whereas non-mycorrhizal fungi (*A. campestris, A. equester, Cantharellus, Craterellus*) contain in sporophores more Cl and less SO_3. If, upon analysis *P. microspora* is found high in S and low in Cl, that would be further evidence that it is mycorrhizal. To control *P. microspora*, fertilizers with Cl rather than with S should be used wherever possible. Finally it may be possible by addition of Mn, S, etc., to spent mushroom compost, to grow edible truffles and other mycorrhizal fungi.

In detecting the truffle disease (62) it is safer to inoculate soil to be tested, directly into tubes of spawn. It has been remarked that compost infested with truffles has odor reminiscent of chlorine (62, 96). On the other hand, it has been shown by use of lead acetate papers that sulfides are emitted from truffle mycelium (175, 177). To the writer the chlorine or quinone odor is typical of spawn itself. Kligman (96) concludes that fungicides are ineffective and that a positive means for controlling truffles lies in maintaining a temperature of 60° F for growing spawn in the beds. His experiments were conducted by exposing spores for one to six hours to fungicides and then filtering off and washing the spores. How can this procedure have any relation to practise? Spores should be sprayed with definite amounts of chemicals, exposed at least 24 hours, and residues left on spores if the procedure is to be a basis for fumigating houses. Neither would it be practical to grow mushroom mycelium at 60° F., when growth is tremendously faster at 75° F., unless a truffle infection exists on the site. Duncan (37, 38) found that by treating compost with one-half pound $CuSO_4$ per ton, there was some indication that such practise might inhibit germination of truffle spores. The writer (185) observed this effect of $CuSO_4$ also but found ten pounds required. With this amount the temperatures during pasteurizing were low, insects were encountered and results were inconclusive. It was noted later that two pounds $CuCl_2$ would suffice, but the $CuCl_2$ was not tested in beds.

Antibiotics and Mechanism of Disease Resistance. There should be some explanation for fungi (*e.g., Mycogone*) attacking the mycelium in the casing soil and not in the compost. The numerous microorganisms in and around the compost are not known to consume the mycelium, although it contains 40% to 50% water-soluble substances. How does the living mycelium resist these molds? The first attempt to account for this "immunity", as it was called, of the mycelium in the compost was made

by Chaze and Sarazin (23). Observing in pure culture that the mushroom mycelium not only prevented but also invaded the mycelium of *Mycogone* and *Verticillium*, they suggest that *A. campestris* has a "defense" due to a "humoral secretion of antibodies". While their explanation is couched in the comparative terminology available at that time, it does account for an active diffusion from the mushroom mycelium that counteracts the toxins of microorganisms producing the same. It is interesting to note that Chaze (22) recently has found an antibiotic from the mycelium of the edible truffle growing in submerged culture.

Undoubtedly the mushroom mycelium has been assayed for antibiotics, but no reference is made to any in a recent review (14) or in a summary of submerged culture (81). In 1944, when investigations on penicillin were in full swing at the University of Wisconsin, the writer requested Mr. O. H. Calvert, who was doing the assay work, to test the liquid

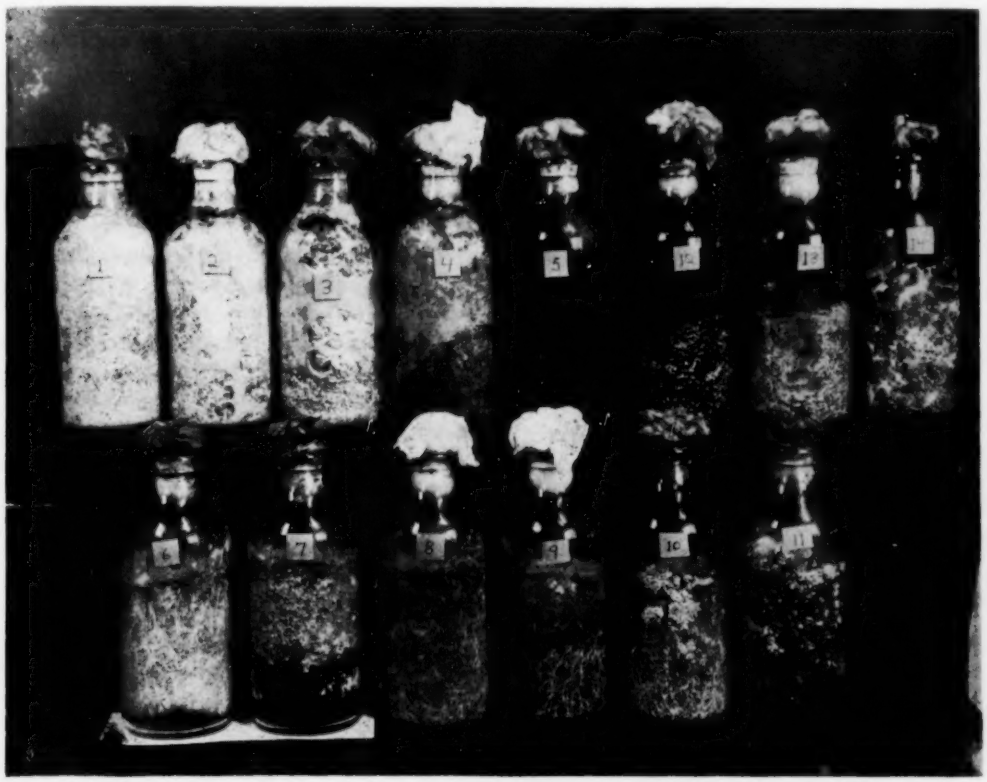

FIG. 5. Truffle disease. 1. Regular spawn-control. 2. Same, inoculated with truffle (T). 3. Later stage. 4. Same, inoculated with nematodes also. 5. Later stage; nematodes did not grow in control 1. 6 to 8. Effect of ammoniation: pasteurized for 72 hrs. at 135° F. 6. Control—previously autoclaved to arrest "urea" bacteria. 7. Same as 6, inoculated T spores. 8. Not autoclaved, inoculated T spores: T spores not germinated—killed by NH_3. 9. Non-sterile root compost; inoculated same time T myc. & spawn. 10. Same as 9, T spores. 11. Same as 9, non-sterile manure compost—spawn growth slight. 12. Root compost autoclaved plus oxalic acid, pH 6.4; T fruit-bodies. 13. Same as 12, no acid, pH 7.1; T mycelium only. 14. T mycelium and fruit-bodies on sterile sorghum grain (185).

media of the mushroom mycelium. Spores germinating in distilled water were transferred to malt extract liquid media and left on the shaking machine until the flasks were full of balls of mycelium. This liquid was completely negative against *Staph. aureus* and *B. coli*. This test, however, is insufficient proof that antibiotics may not be formed by the mycelium because, firstly, it is limited to growth in submerged culture, and secondly, the medium may have been unsuitable. In regard to the first point, Anchel et al. (1) found that *Coprinus similis*, a species closely related to *A. campestris*, produced an antibiotic —5 methoxy-*p*-toluquinone—when grown on a fibrous substrate. Furthermore, they state that it was necessary to extract the substrate with ethyl ether in order to obtain a satisfactory yield and crystals. Secondly, the substrate, as is well known in experiments in the adaptation of enzymes, may be the determining factor for the activity; substrates containing hydroxybenzenes or substances readily converted thereto, or corn steep liquors, may determine the nature and yield of antibiotics formed.

Of the 2191 species of fungi tested for antibiotic activity, 1665 species were Basidiomycetes, and of these 1579 were in the order Agaricales (14). The total number of species found active were 785, of which 549 were among the Agaricales, and of these 258 were in the family Agaricaceae. From among the 549 active species of Agaricales, only 27 antibiotics have been described; and of these the chemical structure of only two are known, one of these being the quinone mentioned (14). Pigments from the sporophores of some fungi have been known for some time to have a quinone structure (86).

It appears, then, that antibiotics are formed considerably among the Agaricaceae, and that antibiotics with a quinone structure have been detected. The antibacterial activities of quinones have been recognized since 1911 (27, 28). There are a number of references to the inhibition of spore germination and antagonism to growth of fungi by quinones, occurring naturally and synthesized chemically (28, 58, 116, 124). Lindeberg (116) suggested the formation of antibiotics by Hymenomycetes through the enzymatic action of extracellular catecholase by oxidation of O-diphenols in the soil, forming quinones which inhibit growth of certain fungi. The oxidases of both the mycelium (63) and sporophore (89) are known to oxidize hydroxy benzenes to quinones. The mycelium in culture on agar has been shown to oxidize hydroxy benzenes (174, 177). Lignin has been mentioned as a source of such compounds. Accordingly it may be proposed that the mechanism of resistance of the mycelium is based on the quinones it forms—" oxidation enzymes are more frequently involved in immunity than we think " (189). Sulfur compounds combine with quinones (27) or copper (prosthetic group of oxidases) (89, 138, 208). In the raging antagonism between *P. microspora* and *A. campestris*, the sulfides which the former produces are probably the deciding element in the struggle.

Insect Pests

Synthetic organic insecticides are important factors contributing to increased yields at the present time. Revised editions of bulletins by experiment stations on biology and control of mushroom insects have been issued (33, 195, 32, 74), but no reference is made to the new insecticides, since these bulletins were published before the war. Comprehensive discussions on the employment of the new insecticides appeared numerously in 1950 (130, 77, 11, 30). New synthetic insecticides are appearing in increasing numbers; two groups are predominant, the chlorinated hydro-carbons (DDT,

BHC, Chlordane, toxaphene) and the organic phosphorous compounds (TEPP, parathion, etc.) (158). DDT came into use first as a mushroom insecticide in the U.S., whereas BHC appears to have been favored in Great Britain, where it originated. DDT is highly effective only against mushroom flies and has a long residual effect. It has the characteristic power of drawing maggots to the surface of the soil, where they may be killed by thiocyanates and rotenone (179). BHC is more volatile than DDT but is effective against all insects infesting mushroom houses (77). Higher gamma isomers of BHC, like " Lindane " which is 99% gamma isomer, does not have the musty odor of BHC (158). Attempts have been made to make the compost uninhabitable for pests by mix-it with insecticides (11, 130). Chlordane and toxaphene appeared to increase yields by spraying before casing (11).

Mites and nematodes are most resistant to insecticides. Considerable study has been devoted to mites (165, 84, 34, 157, 130). The higher gamma isomers of BHC appear to be generally effective against mites on the surface but not in the interior of the bed (157, 130); Lindane has been used also (108-A). The phosphorus insecticides are either too easily hydrolyzed by moisture, or too volatile, or too toxic; their use is limited to applications like spraying floors with parathion. But new miticides are appearing regularly (67). Now that damage by insects is coming under control, it appears that losses by nematodes are becoming more evident. Some species incur, more or less directly, disappearance of mycelium (110); others, while not causing obvious damage, are the agents responsible for reduction of yield (19). Thomas and Mitchell (195-A) have summarized the facts about nematodes. They find that the only satisfactory method for killing nematodes is application of heat; the usual soil sterilization will eradicate nematodes. Sarazin (155-A) observed in preliminary tests that hydroxyquinoline sulfate kills nematodes; at best, though, it is effective only on the surface of the bed.

Submerged Culture

It has been known for a long time that microorganisms, such as yeast, can grow in submerged culture; if the air supply is limited, intermediate products as alcohol, etc., are formed; if aeration is vigorous, the cellular structures are produced abundantly. During the war submerged culture of fungi, especially of *Penicillium* spp., was applied to large-scale production of antibiotics. So it is not surprizing that attempts have been made to grow mushroom mycelium in submerged culture; the advantages, especially as a source of nutrients and flavor for soups, gravies, etc., are obvious.

Humfeld (81) claims to have discovered that *A. campestris* " will grow under conditions of submerged propagation "; this idea may have occurred to him independently, although there are references in the literature that the mycelium of *A. campestris* will grow submerged in a liquid (102, 197). His contribution is, instead, recognition of the commercial possibilities inherent in this feature. Humfeld (81) isolated and tested 40 strains of *A. campestris* in order to find three that were adapted to grow in an agitated aerated liquid medium. His reference to detached cells (secondary spores) as the chief characteristic of strains suitable for submerged growth might be interpreted as referring to strains becoming adapted to an acid medium (pH 4.5). He determined the approximate optimum concentration of various elements and developed a basal medium which contained required amounts of sugar (a wide range of carbohydrates), N (as urea), P, K, Mg, S, Ca, Fe, Mn, Zn and Cu. A comparison of this medium to that of manure shows

that it contains much more N and less K (C : N is 8 : 1; for manure 28 : 1; N : K is 5 : 1; manure 2 : 3); also much more Mg and large amounts of trace elements. Compared to media employed with other fungi for the same proportion of sugar, it contains two or three times as much N, P, K, S, Mg, and 10 to 20 times as much trace elements (168). Yields of mycelium of 60% by weight of sugar consumed were claimed at first (80); later, yields are given as 40% (83), being the same as reported for other fungi (168).

Humfeld (83) states that to obtain mycelium with strong mushroom flavor it is necessary to continue the fermentation one to two days after all the sugar is consumed. The flavor is determined by cooking and tasting. It is doubtful, though, that mycelium has been obtained with a strong mushroom odor; the mild flavor it does have is largely lost in canning. For this reason the many industrial laboratories which joined in the same project after his announcement have discontinued operations. In response to an inquiry of the present status, Humfeld curtly stated that no work is being done at present in government laboratories. The writer was employed by an industrial laboratory where this field was under investigation; while not participating in the program except on the taste panel and in the hunt for new species, it was generally known to all of the laboratory that a mycelium with good flavor was produced from other species, but it was still unsatisfactory. Szuecs (190) has patented a process for extracting essence of the mycelium by steam distillation. Submerged culture certainly has possibilities; considering the funds available for research, it will probably be 10 to 30 years before the method is reduced to practise.

Cultivation of Other Edible Fungi

This topic was discussed at length in the previous review (102). Although there are a number of recent references to cultivation of other edible fungi, in none of them are there cited methods and productivity comparable to that of *A. campestris*. *Morchella esculenta* has been studied intensively in pure culture (15). The rhizomes of Jerusalem artichokes are especially favored by morels; if a planting of morels in a field of artichokes fails, one still has a crop of artichokes (12). Brief references to cultivation of other edible fungi concern *Volvaria volvacea* (75), *Pholiota aergerito* (75), morel (75), truffle (149), *Cortinellus shiitake* (92), *Cortinelus berkeleyanus* (134), *Tricholoma conglobatum* (133), *Collybia veluptes* (118, 9), *Armellaieu melles* (118, 9) *Pleurotus ostreatus* (118, 9) and mushroom growing in India (136).

References to the possibilities of cultivating mycorrhizal fungi has been made. Dr. E. B. Lambert informed many in the mushroom industry some ten years ago that he obtained a crop of about eight pounds per square foot with *Coprinus comatus*, but in later attempts he could not repeat this feat. The writer obtained a culture from him and found that the mycelium grew vigorously on synthetic straw compost. The fructification was, as Lambert found, a puzzle; at times no sporophores developed; at best, one or two pounds per square foot were obtained. Recent studies on the sex and the "intersterility barriers that divide the strains" should lift the barrier to the cultivation of this mushroom (112, 209).

Nutritive Value of Mushrooms

The comprehensive bulletin issued by the Massachusetts Agricultural Experiment Station on the nutritive properties of mushrooms (50) should help to dispel the popular notion that the mushroom is merely a flavoring material. It does possess definite food values. This bulletin by Esselen and Fellers (50) represents a summation of previous reports over a period of five years from this Sta-

tion (2, 51, 52, 123). The mineral analysis (2) closely resembles that of Friese (60); Cu is present as might be expected from its function in the oxidases. In rat-feeding experiments the nutritive value of the mushroom proteins was found to be 56.6% (52). This amount is less than was detected in earlier analyses; but in recent tests with human beings the digestibility of proteins is given as 72% to 83% (117). All the essential amino acids are present, but the tryptophane content is particularly low. The surprising characteristic of the composition of carbohydrates is the large amount of mannitol (123). Mushrooms are especially rich in the B vitamins, particularly riboflavin, nicotinic and folic acids, but do not contain any vitamin A, D or E (2). They also lack vitamin B_{12}, although it is present in composted manure (128).

An interesting investigation was pursued on the vitamin content of canned mushrooms (51). The vitamins analyzed were riboflavin, nicotinic and pantothenic acids and biotin; retention of these vitamins after canning and storage for two months was 78% to 85%; after six to 12 months, 60% to 70%. They conducted analyses of canned mushrooms from six different canners and found that commercially canned mushrooms, as purchased by consumers, were an excellent source of the B vitamins. They conclude that fresh mushrooms contain approximately 2.67% proteins; while mushrooms are not comparable with meat as a source of protein, they compare favorably with many fresh vegetables. The mushroom has immense possibilities as a converter of roughages; with continued research, it may some day rival stock animals.

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Note { M.G.A. = Mushroom Growers' Association
M.R.A. = Mushroom Research Association
M.R.S. = Mushroom Research Station

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Utilization Abstracts

Natural Candy Flavors. These products consist of extracts, essences, pastes and oils derived from a variety of plants and various parts thereof. The flavor manufacturer separates the flavoring principles from the extraneous matter by expression, maceration, percolation and distillation. Among them are vanilla extract from vanilla beans; maple concentrates, "usually made by desugaring maple syrup to an extent where the maple flavor becomes the predominant factor instead of sweetness"; coffee concentrates, "prepared by extracting ground coffee with a menstruum and concentration of the extract by distillation under vacuum"; licorice extract, "made by percolating coarsely ground licorice root with boiling distilled water until the root is exhausted. The percolate is then concentrated to about one-half of its original volume by boiling under normal atmospheric pressure and then filtered. After filtration the water content of the filtrate is evaporated until the residue does not contain more than 17% of water"; malt extract, "prepared by infusing malt with water at 60° Centigrade, concentrating the expressed liquid at a temperature not exceeding 60° C. and adding 10% glycerin by weight"; fruit pastes or purees, "made by comminuting fruit to a paste with addition of sugar in the ratio of 3 to 5 parts of fruit to one part of sugar"; fruit flavor concentrates, "essentially fruit juices whose moisture content has been largely eliminated and partly replaced by alcohol and/or sugar. This elimination is done either by distillation under high vacuum or in the case of fruit sensitive to heat by freezing out the water and separating the concentrated juice from the ice particles by centrifuging"; and concentrated citrus oils, expressed from the peel of oranges, lemons and grapefruits. (Paul Adams, *Am. Perf. & Ess. Oil Rev. 60: 130. 1952*).

Oak Moss. Oak moss [principally European species of the lichen genera *Ramelina* and *Evernia*] in Europe is the source of several perfumery products, known to the trade as tinctures, concrete and absolute oils, resinoids, liquid oils, decolorized and colorless oils, and distilled oils. The "moss" gathered from oak trees provides the best oils, whereas that from conifers, known as "treemoss oils" or "essences de mousse d'arbre", is inferior, and iodine is added to it for improvement. (F. Chatelain, *Perf. & Essen. Oil Rec. 44*(3): *91. 1953*).

BOOK REVIEWS

Cocoa—Cultivation, Processing, Analysis. Eileen M. Chatt. xvi + 302 pages. Interscience Publishers, Inc. 1953. $8.50.

In 1949 Interscience Publishers began a new series of books, known as "Economic Crops" and designed to be of interest to the academically minded reader as well as to the technologist engaged in producing, processing and marketing such crops in their various forms of utilization. The first two volumes, on bananas and apples, respectively, were briefly reviewed in the October–December, 1950, issue of ECONOMIC BOTANY. Very recently the third volume, this time on cacao, has been published, and here again the biologist, but especially the manufacturer, finds a wealth of information on the history, botany and exploitation of an important crop plant.

Cocoa is one of the vegetable gifts of America to the foods of man. Columbus, on his fourth voyage in 1502, was the first European to see a cargo of the beans. He introduced them into Spain, but only as a souvenir, and it was not until more than a century later, in 1632, that a publication in Europe gave any record of their importance. That was in the account of the historian who had accompanied Cortes on his conquest of Mexico 65 years earlier. Therein was recorded for posterity the oft told tale of 50 jars of chocolate beverage being prepared for daily consumption by Montezuma, the Aztec emperor of Mexico, and of 2,000 additional jars for his household. So highly esteemed were the beans that at one time 100 of them constituted the price of a slave.

Introduced into Europe by the Spaniards, chocolate became very popular. At the French court it developed into the fashionable drink, and somewhere in the eighteenth century a French artist painted a portrait of a chocolate server which his long hung in the famous Dresden Art Gallery and which, about 80 years ago, became the original of the famous trademark of Walter Baker & Co., chocolate manufacturers of Dorchester, Mass.

Today cacao is grown in and exported from more than 50 countries, and total world production has increased from about 80,000 metric tons in 1895 to perhaps 700,000 tons today. Eight modern cacao products are recognized and defined by the U. S. Food and Drug Administration—cacao nibs, chocolate liquor, cocoa powder, low fat cocoa, sweetened cocoa powder, cocoa butter, chocolate and milk chocolate. Cocoa butter consists of the fat content pressed out of the seeds or "nibs", and the percentage of this fat left in or added to the residue after extraction is important in manufacturing eating chocolate and beverage cocoa. Manufacture of all these products is accomplished through modern technology and agricultural practice, details of which are extensively considered in this book.

Algal Culture—From Laboratory to Pilot Plant. Edited by John S. Burlew. 357 pages. Carnegie Inst. Washington, Publ. 600. 1953.

The present world population of about two billion human beings is expected to triple within the next hundred years. Accompanying this growth will be the ever increasing problem of providing food for human consumption, particularly fats, oils and proteins. A few individuals and institutions are already endeavoring in a very imaginative but scientific manner to contribute toward averting future starvation in threatened parts of the world. This latest and as yet purely experimental approach was initiated by the discovery in 1942 at the Department of Plant Biology, Carnegie Institution of Washington, at Stanford, California, that growth of the microscopic alga *Chlorella* can be controlled to produce cells of either a high fat or a high protein content. The possibilities of enormous practical value inherent in this discovery have been investigated under various auspices in the United States and also in England, Germany, Venezuela, Israel and Japan. The results of all these investigations, along with a very readable account of algal biology and utilization in general, are presented in this Publication of the Carnegie Institution of Washington which has been so prominent in initiating and furthering this work.